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Determination of Drug Pharmacokinetics  
and Metabolic Profile

Volume I

ANNUAL AND FINAL REPORT

March 1, 1988

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<p>→ The pharmacokinetics and metabolism of halofantrine, WR 171,669, have been studied in beagle dogs after oral doses of 5 to 100 mg/kg of the carbon-14 labelled drug. The routes and rates of excretion and plasma and blood concentrations of the drug and its metabolites were measured. A similar investigation was carried out with WR 178,460, the N-desbutyl derivative of WR 171,669, administered as a single oral dose of 21 mg/kg to dogs.</p> <p>Rates and routes of excretion and plasma and blood concentrations of WR 158,122 and its metabolites have been investigated in rhesus monkeys after administration of 10 mg/kg oral doses to animals with cannulated bile ducts. Metabolites were quantified after chromatographic separation and information on their structure obtained by mass spectrometry of isolated samples. <i>Keywords: pyridoxamine, halofantrine, inhibition, label, substance, halofantrine hydrochloride</i></p>					
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# FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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## INTRODUCTION

During the period of this contract from November 1982 to September 1986 we have been involved in various studies on bioavailability, pharmacokinetics and metabolism of drugs in animals. This work has included studies on the pharmacokinetics and metabolism of four different compounds WR 171,669, WR 178,460, WR 158,122 and WR 238605 all new potential anti-malarial drugs. These studies have been carried out with beagle dogs except for WR 158,122 which was studied in rhesus monkeys and WR 238605 where dogs and rhesus monkeys were used.

In addition two bioavailability and pharmacokinetic studies with pyridostigmine have been completed in beagle dogs. These studies were designed to assess the bioavailability of pyridostigmine when administered in tablet and syrup formulations.

METABOLISM AND PHARMACOKINETICS OF  
<sup>14</sup>C-HALOFANTRINE HYDROCHLORIDE  
(<sup>14</sup>C-WR 171,669.HCl) IN THE DOG  
(PILOT STUDY)

## SUMMARY

1. The purpose of this study was to carry out a pilot metabolism and pharmacokinetic study of the new potential antimalarial drug  $^{14}\text{C}$ -WR 171,669.HCl in the beagle dog, which would assist in the design and analysis of samples in a more extensive main study. In this study the drug was administered orally, as a suspension in a 0.4% aqueous solution of Tween 80 containing 0.2% (w/v) methylcellulose, at a dose level of 15 mg/kg bodyweight.
2. Almost all the radioactive dose was excreted in the faeces, 96.4% during 7 days. Most of this material was eliminated during the first 48 hours (72.8%). During the seventh day, only 1.1% was excreted in the faeces. A total of only 0.1% of the dose was measured in urine.
3. Peak plasma concentrations of radioactivity occurred at 4 to 5 hours (0.95  $\mu\text{g/ml}$ ) declined up to 12 hours and then increased to a secondary peak at 24 hours (0.71  $\mu\text{g/ml}$ ). Concentrations then appeared to decline biphasically with an initial rapid phase until 7 days ( $t_{1/2}$  37 hours) and a slower terminal phase ( $t_{1/2}$  502 hours). Blood concentrations of radioactivity followed a similar profile and were always lower than plasma.
4. Almost all the radioactivity (86%) in a solvent extract of the 0-24 hour faeces sample was associated with the unchanged drug. The remaining radioactivity was associated with several more polar components.

A chromatographic method was developed using reverse phase hplc for the separation of WR 171,669 and its metabolites in plasma.

The concentration of the unchanged drug in plasma declined from 0.49  $\mu\text{g/ml}$  at 3 hours to 0.13  $\mu\text{g/ml}$  at 30 hours. During this time the proportion of WR 171,669 declined from 55% of total radioactivity at 3 hours to 22% at 30 hours with a corresponding increase in the amounts of some more polar metabolites. These metabolites did not seem to correspond chromatographically with N-desbutyl WR 171,669 (WR 173,460) under the test conditions.

TABLE 1

Excretion of radioactivity after oral administration of  
 $^{14}\text{C}$ -WR 171,669.HCl to a beagle dog at a dose level of 15 mg/kg

Time (hours)	Radioactivity excreted (% dose)			Cumulative excretion (% dose)			
	Urine	Faeces	Cage wash	Urine	Faeces	Cage wash	Total
0- 24	0.04	52.1	2.3	0.04	52.1	2.3	54.4
24- 48	0.03	20.7	0.6	0.07	72.8	2.9	75.8
48- 72	0.01	15.2	0.1	0.08	88.0	3.0	91.1
72- 96	0.01	4.5	0.1	0.09	92.5	3.1	95.7
96-120	0.01	1.6	<0.1	0.10	94.1	3.1	97.3
120-144	<0.01	1.2	0.1	0.10	95.3	3.2	98.6
144-168	0.01	1.1	<0.1	0.11	96.4	3.2	99.7



TABLE 2

Concentrations of radioactivity in plasma and whole-blood after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl to a beagle dog at a dose level of 15 mg/kg

Time	$\mu\text{g}$ equivalents WR 171,669.HCl/ml		% dose/litre	
	Plasma	Whole-blood	Plasma	Whole-blood
15 mins	<0.08	<0.05	<0.05	<0.03
30 mins	0.12	0.08	0.07	0.05
45 mins	0.24	0.17	0.15	0.11
1 hrs	0.45	0.34	0.28	0.21
2 hrs	0.72	0.53	0.45	0.33
3 hrs	0.90	0.68	0.57	0.43
4 hrs	0.94	0.72	0.59	0.45
5 hrs	0.95	0.73	0.59	0.46
7 hrs	0.83	0.69	0.52	0.43
12 hrs	0.60	0.53	0.38	0.33
24 hrs	0.71	0.55	0.44	0.35
30 hrs	0.61	0.52	0.38	0.33
2 days	0.54	0.42	0.34	0.26
3 days	0.33	0.24	0.21	0.15
4 days	0.20	0.12	0.12	0.08
5 days	0.15	0.09	0.10	0.06
6 days	0.11	0.07	0.07	0.04
7 days	0.07	<0.06	0.05	<0.04
8 days	0.07	<0.06	0.04	<0.04
10 days	0.05	<0.06	0.03	<0.04
12 days	0.05	<0.06	0.03	<0.04
14 days	0.04	<0.06	0.02	<0.04
19 days	0.03	<0.06	0.02	<0.04

TABLE 3

Proportions of radioactivity in methanol extracts of faeces samples  
up to 72 hours after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl  
to a beagle dog at a dose level of 15 mg/kg

Results are expressed as % faecal radioactivity

Time period (hours)	Extract 1	Extract 2*	Total
0-24	68.0	15.9	83.9
24-48	54.8	10.4	65.2
48-72	27.6	21.9	49.5

\* Methanol : diethylamine (9 : 1, v/v)

TABLE 4

Radioactivity in 1 minute fractions of hplc eluate after injection of extracts of faeces collected during 24 hours after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl to a beagle dog at a dose level of 15 mg/kg

Fraction numbers (inclusive)	% eluted radioactivity		Total % dose (Extracts 1 and 2)
	Extract 1	Extract 2	
2- 9	7.0	21.7	4.3
16-25	6.6	-	2.3
27-35*	86.0	78.3	37.0

\* Corresponds to elution of  $^{14}\text{C}$ -WR 171,669

TABLE 5

Concentrations of  $^{14}\text{C}$ -WR 171,669 free base\* in plasma after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl to a beagle dog at a dose level of 15 mg/kg

Time (hrs)	Concentration ( $\mu\text{g/ml}$ )*	% eluted radioactivity
3	0.46	54.5
7	0.39	50.1
12	0.18	32.5
30	0.12	21.6

\* Refers to radioactivity eluted from hplc column with the same retention time as authentic  $^{14}\text{C}$ -WR 171,669  
See Table 6. Results are not corrected for recovery



FIGURE 1

Concentrations of radioactivity in plasma (o-o) and whole blood (●-●) after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl to a beagle dog

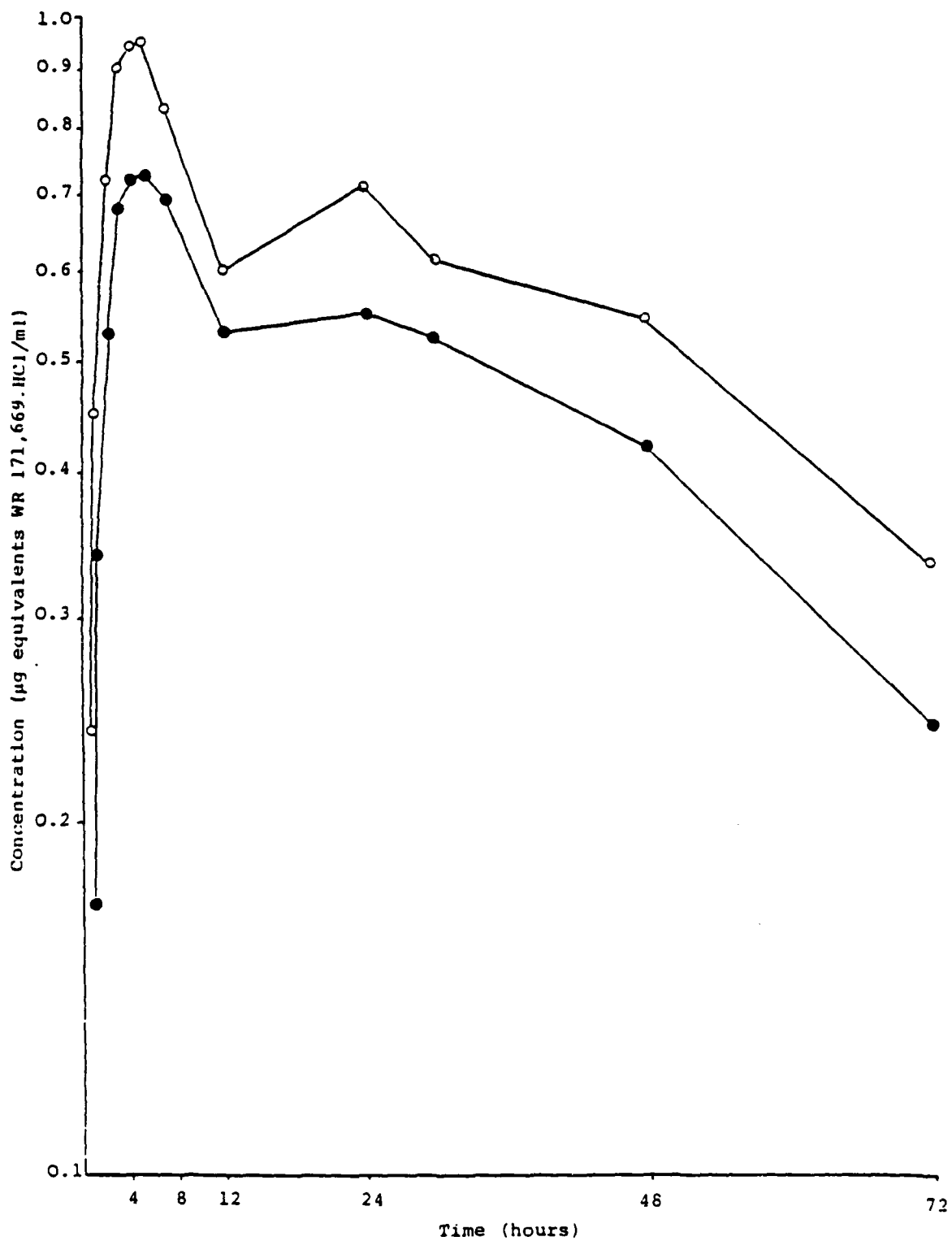
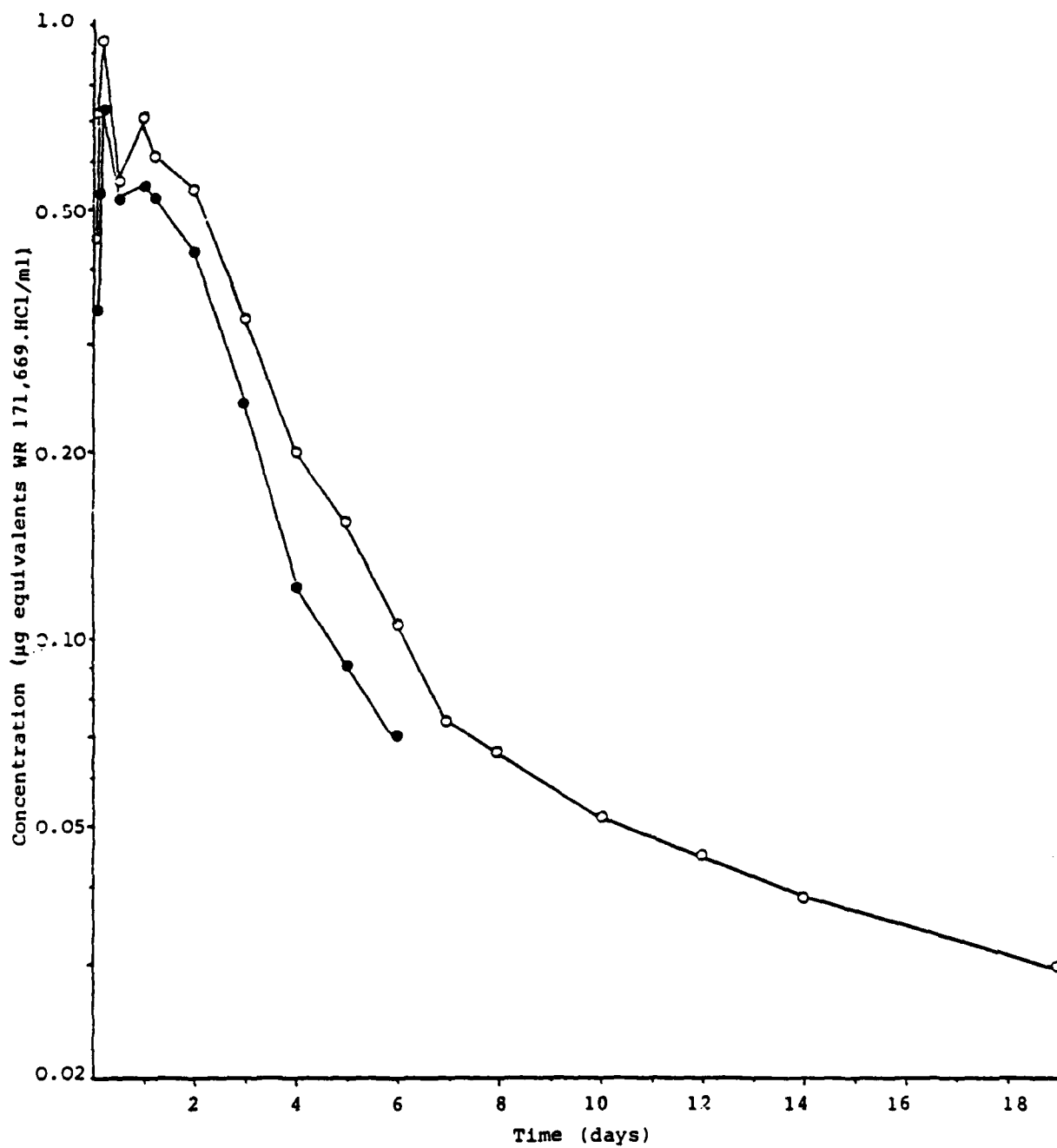


FIGURE 2

Concentrations of radioactivity in plasma (o-o) and whole blood (●-●) after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl to a beagle dog



METABOLISM AND PHARMACOKINETICS OF  
<sup>14</sup>C-HALOFANTRINE HYDROCHLORIDE  
(<sup>14</sup>C-WR 171,669.HCl) IN THE DOG  
(MAIN STUDY)



## SUMMARY

1. The purpose of this study was to investigate the metabolism and pharmacokinetics in the beagle dog of the new potential anti-malarial drug, WR 171,669.HCl. Single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl have been administered to six dogs, each dog receiving the drug at a different dose level within the range 5 to 100 mg/kg. The drug was administered in suspension in 1% aqueous sodium carboxymethylcellulose solution. The radiolabelled drug was shown to be stable under the conditions of dosing. No clinical or other drug-related effects were observed in the dogs after dosing.
2. Excretion of radioactivity in faeces accounted for between 75.3% and 89.2% dose during seven days after administration. Urinary excretion during seven days ranged from 0.03% dose (100 mg/kg dose) to 0.31% dose (5 mg/kg dose).
3. Peak plasma concentrations of radioactivity (0.39 - 2.01  $\mu\text{g}$  equiv. free base/ml) occurred at times from 1 to 30 hours after dosing. Radioactivity could be detected in the plasma of all animals at 10 days after dosing and at 24 days in the plasma from animals dosed at 40 or 60 mg/kg. Total areas under plasma radioactivity concentration-time curves increased in an approximately linear fashion with dose level from 5 to 60 mg/kg, but the result obtained from the 100 mg/kg dose level was 5 times lower than expected.
4. Whole-blood radioactivity concentrations similar to or higher than in plasma occurred at some sampling times after dosing at 5, 40 or 100 mg/kg, (maximum ratio 1.9), but not after dosing at 10, 20 or 60 mg/kg.

5. Peak concentrations of unchanged WR 171,669 in plasma (0.30 - 1.39  $\mu\text{g/ml}$ ) occurred at 1 to 2 hours (5, 10, 20, 100 mg/kg) or 5 - 6 hours (40 and 60 mg/kg). Second peaks occurred at 30 and 24 hours after the 10 and 60 mg/kg doses. The area under the concentration-time curve showed an approximately linear increase with dose level up to 60 mg/kg but was about 5 times lower than expected at 100 mg/kg. Estimated half-lives for the decline in concentrations were in the range 4.3 - 8.0 hours for animals showing a single concentration maximum and 20 and 25 hours for the other two animals.
6. The proportion of plasma radioactivity associated with metabolites of WR 171,669 in plasma increased with time after dosing. Metabolites were eluted with shorter retention times than WR 171,669 under reverse phase hplc conditions. A minor metabolite had the same retention time as desbutyl WR 171,669 (WR 178,460).
7. Unchanged WR 171,669 was the major component in extracts of 0-24 hour faeces but a minor component in most later extracts. In extracts up to 72 hours, WR 171,669 accounted for 17% dose (5 mg/kg) to >40% dose (40, 60, 100 mg/kg). A minor radioactive component in faecal extracts corresponded chromatographically (hplc and tlc) to WR 178,460. Thin-layer chromatography showed the presence of a number of other radioactive metabolites in faeces extracts. A similar pattern of radioactive metabolites was found in extracts of faeces from an animal which had received  $^{14}\text{C}$ -WR 178,460.HCl suggesting the existence of common pathways of metabolism for the two compounds.

TABLE 1

Excretion of radioactivity by dogs after single oral doses of  
<sup>14</sup>C-WR 171,669.HCl at various levels  
 Results are expressed as % administered radioactivity

Sample	Time period (hours)	Nominal dose level (mg/kg)					
		5	10	20	40	60	100
<u>Urine</u>	0 - 6	<0.01	<0.01	<0.01	NS	<0.01	<0.01
	6 - 24	0.09	0.06	0.05	0.03	0.02	0.02
	24 - 48	0.09	0.08	0.03	0.02	0.03	0.01
	48 - 72	0.04	0.03	0.02	0.01	0.01	<0.01
	72 - 96	0.03	0.04	0.06	0.01	0.02	<0.01
	96 - 120	0.03	0.01	0.01	<0.01	0.01	<0.01
	120 - 144	0.02	0.01	0.01	<0.01	0.01	<0.01
	144 - 168	0.01	<0.01	0.01	0.01	<0.01	<0.01
	Total	0.31	0.23	0.19	0.08	0.10	0.03
<u>Faeces</u>	0 - 24	43.7	22.8	55.3	55.5	60.0	84.3
	24 - 48	15.5	24.8	12.1	15.2	7.1	1.7
	48 - 72	13.3	11.9	7.3	3.8	NS	0.9
	72 - 96	6.1	6.4	4.2	3.7	1.8	0.7
	96 - 120	5.6	4.8	2.2	2.5	6.2	0.6
	120 - 144	2.8	3.3	2.2	2.2	3.5	0.5
	144 - 168	2.2	1.3	NS	1.1	1.5	0.3
	Total	89.2	75.3	83.3	84.0	80.1	89.0
<u>Cagewash</u>	0 - 24	0.26	0.32	0.06	0.24	0.08	0.20
	24 - 48	0.06	0.23	0.09	0.09	0.08	0.09
	48 - 72	0.14	0.17	0.24	0.07	0.08	0.04
	72 - 96	0.08	0.15	0.18	0.02	0.02	<0.01
	96 - 120	0.09	0.08	0.06	0.03	0.09	0.01
	120 - 144	0.04	0.02	0.06	0.03	0.05	<0.01
	144 - 168	0.03	0.03	0.02	0.05	0.03	<0.01
	Total	0.70	1.00	0.71	0.53	0.43	0.34
Overall total		90.2	76.5	84.2	84.6	80.6	89.4

NS No sample

TABLE 2

Cumulative excretion of radioactivity by dogs after single oral doses of  
 $^{14}\text{C}$ -WR 171,669.HCl at various levels  
 Results are expressed as % administered radioactivity

Sample	Time period (hours)	Nominal dose level (mg/kg)					
		5	10	20	40	60	100
<u>Urine</u>	0 - 6	<0.01	<0.01	<0.01	NS	<0.01	<0.01
	0 - 24	0.09	0.06	0.05	0.03	0.02	0.02
	0 - 48	0.18	0.14	0.08	0.05	0.05	0.03
	0 - 72	0.22	0.17	0.10	0.06	0.06	0.03
	0 - 96	0.25	0.21	0.16	0.07	0.08	0.03
	0 - 120	0.28	0.22	0.17	0.07	0.09	0.03
	0 - 144	0.30	0.23	0.18	0.07	0.10	0.03
	0 - 168	0.31	0.23	0.19	0.08	0.10	0.03
<u>Faeces</u>	0 - 24	43.7	22.8	55.3	55.5	60.0	84.3
	0 - 48	59.2	47.6	67.4	70.7	67.1	86.0
	0 - 72	72.5	59.5	74.7	74.5	67.1	86.9
	0 - 96	78.6	65.9	78.9	78.2	68.9	87.6
	0 - 120	84.2	70.7	81.1	80.7	75.1	88.2
	0 - 144	87.0	74.0	83.3	82.9	78.6	88.7
	0 - 168	89.2	75.3	83.3	84.0	80.1	89.0
<u>Cagewash</u>	0 - 24	0.26	0.32	0.06	0.24	0.08	0.20
	0 - 48	0.32	0.55	0.15	0.33	0.16	0.29
	0 - 72	0.46	0.72	0.39	0.40	0.24	0.33
	0 - 96	0.54	0.87	0.57	0.42	0.26	0.33
	0 - 120	0.63	0.95	0.63	0.45	0.35	0.34
	0 - 144	0.67	0.97	0.69	0.48	0.40	0.34
	0 - 168	0.70	1.00	0.71	0.53	0.43	0.34
Overall total		90.2	76.5	84.2	84.6	80.6	89.4

NS No sample

TABLE 3

Concentrations of radioactivity in plasma of dogs after single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl at various levels  
Results are expressed as  $\mu\text{g}$  equivalents WR 171,669 free base/ml

Time	Nominal dose level (mg/kg)					
	5	10	20	40	60	100
0.5 hour	0.08	0.19	0.71	0.10	0.09	0.25
1	0.23	0.37	1.62	0.32	0.30	0.58
2	0.39	0.46	1.52	0.95	0.69	0.82
3	0.39	0.52	1.41	1.26	1.09	0.93
4	0.38	0.53	1.29	1.76	1.43	0.85
5	0.38	0.53	1.21	1.99	1.55	1.00
6	0.38	0.54	1.09	2.01	1.58	0.93
7	0.37	0.52	1.04	1.87	1.48	0.91
12	0.23	0.65	0.90	1.35	1.26	0.67
24	0.17	0.36	0.59	0.89	1.83	0.51
30	0.16	0.70	0.50	0.72	1.36	0.45
2 days	0.14	0.56	0.50	0.69	1.00	0.32
3	0.11	0.43	0.43	0.67	0.86	0.26
4	0.11	0.32	0.32	0.66	1.04	0.23
5	0.08	0.22	0.24	0.51	0.71	0.20
6	0.05	0.14	0.17	0.33	0.63	0.19
7	0.04	0.10	0.13	0.25	0.53	0.16
8	0.03	0.08	0.10	0.21	0.43	0.16
10	0.02	0.05	0.07	0.14	0.26	0.12
12	<0.02	0.04	0.05	0.11	0.19	0.11
15	<0.02	0.03	0.04	0.08	0.12	0.08
18	<0.02	<0.02	0.03	0.06	0.09	0.05
21	<0.02	<0.02	<0.02	0.05	0.05	<0.04
24	<0.02	<0.02	<0.02	0.05	0.04	<0.04
28	<0.02	<0.02	<0.02	<0.04	<0.04	<0.04

TABLE 4

Concentrations of radioactivity in plasma of dogs after single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl at various levels  
Results are expressed as % dose/litre

Time	Nominal dose level (mg/kg)					
	5	10	20	40	60	100
0.5 hour	0.13	0.15	0.30	0.02	0.01	0.022
1	0.35	0.30	0.68	0.06	0.04	0.050
2	0.61	0.37	0.64	0.19	0.10	0.071
3	0.61	0.42	0.59	0.26	0.16	0.081
4	0.59	0.42	0.54	0.36	0.21	0.074
5	0.58	0.43	0.51	0.40	0.23	0.087
6	0.59	0.43	0.46	0.41	0.23	0.081
7	0.57	0.42	0.44	0.38	0.22	0.079
12	0.36	0.52	0.38	0.27	0.18	0.058
24	0.27	0.29	0.25	0.18	0.27	0.044
30	0.25	0.56	0.21	0.15	0.20	0.040
2 days	0.21	0.45	0.21	0.14	0.15	0.028
3	0.18	0.34	0.18	0.14	0.12	0.023
4	0.17	0.25	0.13	0.13	0.15	0.020
5	0.12	0.17	0.10	0.10	0.10	0.017
6	0.08	0.11	0.07	0.07	0.09	0.016
7	0.07	0.08	0.06	0.05	0.08	0.014
8	0.05	0.06	0.04	0.04	0.06	0.014
10	0.03	0.04	0.03	0.03	0.04	0.011
12	<0.03	0.03	0.02	0.02	0.03	0.010
15	<0.03	0.02	0.02	0.02	0.02	0.007
18	<0.03	<0.02	0.01	0.01	0.01	0.004
21	<0.03	<0.02	<0.01	0.01	0.008	<0.004
24	<0.03	<0.02	<0.01	0.009	0.006	<0.004
28	<0.03	<0.02	<0.01	<0.008	<0.006	<0.004

TABLE 5

Concentrations of radioactivity in whole-blood of dogs after single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl at various levels. Results are expressed as  $\mu\text{g}$  equivalents WR 171,669 free base/ml

Time	Nominal dose level (mg/kg)					
	5	10	20	40	60	100
0.5 hour	0.05	0.12	0.52	<0.10	<0.10	0.17
1	0.17	0.25	1.12	0.23	0.20	0.44
2	0.32	0.33	1.16	0.72	0.52	0.69
3	0.33	0.38	1.12	0.97	0.84	0.82
4	0.31	0.37	1.03	1.20	1.14	0.72
5	0.31	0.35	0.90	1.47	1.16	0.80
6	0.33	0.38	0.87	1.53	0.84	0.79
7	0.29	0.37	0.80	1.38	1.08	0.76
12	0.24	0.28	0.68	1.09	0.83	0.55
24	0.17	0.25	0.50	0.76	1.46	0.47
30	0.16	0.52	0.36	0.88	1.14	0.53
2 days	0.13	0.43	0.34	0.81	0.86	0.37
3	0.11	0.32	0.30	0.61	0.73	0.35
4	0.09	0.21	0.26	0.44	0.81	0.33
5	0.07	0.15	0.16	0.21	0.60	0.28
6	<0.05	0.09	0.11	0.28	0.44	0.25
7	<0.05	0.07	0.10	0.21	0.41	0.23
8	<0.05	0.07	0.07	0.18	0.32	0.26
10	<0.05	<0.05	<0.05	0.11	0.20	0.19
12	<0.05	<0.05	<0.05	<0.10	0.12	0.16
15	<0.05	<0.05	<0.05	<0.10	0.10	0.15
18	<0.05	<0.05	<0.05	<0.10	<0.10	<0.10
21	<0.05	<0.05	<0.05	<0.10	<0.10	<0.10
24	<0.05	<0.05	<0.05	<0.10	<0.10	<0.10
28	<0.05	<0.05	<0.05	<0.10	<0.10	<0.10

TABLE 6

Concentrations of radioactivity in whole-blood of dogs after single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl at various levels  
Results are expressed as % dose/litre

Time	Nominal dose level (mg/kg)					
	5	10	20	40	60	100
0.5 hour	0.08	0.10	0.22	<0.02	<0.02	0.015
1	0.27	0.20	0.47	0.05	0.03	0.038
2	0.50	0.26	0.49	0.15	0.08	0.060
3	0.51	0.30	0.47	0.20	0.12	0.071
4	0.48	0.30	0.44	0.24	0.17	0.062
5	0.49	0.28	0.38	0.30	0.17	0.070
6	0.51	0.31	0.37	0.31	0.12	0.069
7	0.46	0.30	0.34	0.28	0.16	0.066
12	0.37	0.22	0.29	0.22	0.12	0.048
24	0.26	0.20	0.21	0.15	0.21	0.041
30	0.25	0.41	0.15	0.18	0.17	0.046
2 days	0.20	0.34	0.15	0.16	0.13	0.032
3	0.17	0.25	0.13	0.12	0.11	0.031
4	0.14	0.16	0.11	0.09	0.12	0.029
5	0.10	0.12	0.07	0.04	0.09	0.024
6	<0.09	0.07	0.05	0.06	0.06	0.022
7	<0.09	0.05	0.04	0.04	0.06	0.020
8	<0.09	0.06	0.03	0.04	0.05	0.023
10	<0.09	<0.05	<0.03	0.02	0.03	0.016
12	<0.09	<0.05	<0.03	<0.02	0.02	0.014
15	<0.09	<0.05	<0.03	<0.02	0.02	0.013
18	<0.09	<0.05	<0.03	<0.02	<0.02	<0.010
21	<0.09	<0.05	<0.03	<0.02	<0.02	<0.010
24	<0.09	<0.05	<0.03	<0.02	<0.02	<0.010
28	<0.09	<0.05	<0.03	<0.02	<0.02	<0.010



TABLE 7

Total areas under plasma radioactivity concentration -  
time curves after single oral doses  
of  $^{14}\text{C}$ -WR 171,669.HCl to dogs  
at various levels

Dose level (mg/kg)	Area ( $\mu\text{g h.ml}^{-1}$ )	Area/dose level ( $\mu\text{g h ml}^{-1} \text{mg}^{-1} \text{kg}$ )
5.01	23.46	4.7
10.2	72.19	7.1
19.6	86.21	4.4
40.8	152.29	3.7
61.4	227.52	3.7
102.7	82.74	0.80

TABLE 8

Concentrations of WR 171,669\* free base in plasma of dogs  
after single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl at various levels  
Results are expressed as  $\mu\text{g/ml}$

Time (hours)	Nominal dose level (mg/kg)					
	5	10	20	40	60	100
0.5	NM	NM	0.66	NM	NM	NM
1	0.23	0.34	1.27	0.06	0.07	0.53
2	0.30	0.25	1.19	0.77	0.50	0.73
3	0.25	0.22	0.96	1.01	0.71	0.71
4	0.20	0.20	0.80	1.27	0.93	0.58
5	0.21	0.17	0.65	1.39	0.89	0.64
6	0.19	0.20	0.55	1.33	0.95	0.60
7	0.18	0.17	0.32	1.25	0.83	0.64
12	0.06	0.08	0.28	0.72	0.45	0.26
24	<0.03	0.08	0.09	0.27	1.02	0.11
30	NM	0.17	0.04	0.19	0.59	0.07
48	NM	0.07	<0.03	0.07	0.25	NM
72	NM	<0.03	<0.03	<0.05	0.10	NM
96	NM	<0.03	<0.06	<0.05	0.06	NM
120	NM	<0.04	<0.06	<0.05	<0.06	NM
144	NM	NM	NM	NM	<0.06	NM
168	NM	NM	NM	NM	<0.07	NM

NM Not measured

\* Refers to radioactivity eluting from an hplc column  
with the same retention time as WR 171,669. Results  
are not corrected for recovery

Calculated limits of detection are based on the limit  
of detection for a single fraction of hplc eluate  
(Appendix 5)

TABLE 9

Total areas under plasma concentrations of WR 171,669 free base  
against time curves after single oral doses of  
of  $^{14}\text{C}$ -WR 171,669.HCl to dogs  
at various levels

## (a) Observed curves

Dose level (mg/kg)*	Area $\dagger$ ( $\mu\text{g h.ml}^{-1}$ )	Area/dose level ( $\mu\text{g h.ml}^{-1} \text{mg}^{-1} \text{kg}$ )
4.67	2.07	0.44
9.51	5.96	0.63
18.3	9.70	0.53
38.0	21.04	0.55
57.2	35.00	0.61
95.7	9.12	0.095

\* Calculated as WR 171,669 free base

$\dagger$  To time of last detectable measurement

## (b) Fitted curves (see Table 19)

Dose level (mg/kg)*	Area $\#$ ( $\mu\text{g h.ml}^{-1}$ )	Area/dose level ( $\mu\text{g h.ml}^{-1} \text{mg}^{-1} \text{kg}$ )
4.67	2.48	0.53
9.51	6.49	0.68
18.3	8.40	0.46
38.0	21.11	0.56
57.2	34.11	0.60
95.7	9.64	0.10

# To infinite time

TABLE 10

Concentrations of radioactivity in plasma of dogs (after oral doses of  $^{14}\text{C}$ -WR 171,669.HCl) associated with a component eluting from an hplc column with the same retention time as WR 178,460  
Results are expressed as  $\mu\text{g}$  equivalents WR 178,460 free base/ml

Time (hours)	Nominal dose level (mg/kg)					
	5	10	20	40	60	100
0.5	NM	NM	+	NM	NM	NM
1	+	+	+	+	+	+
2	0.02	0.02	0.10	0.04	+	0.04
3	0.02	0.04	0.12	0.05	0.12	0.09
4	0.03	0.06	0.14	0.11	0.15	0.12
5	0.04	0.07	0.14	0.18	0.21	0.17
6	0.05	0.08	0.14	0.21	0.27	0.17
7	0.06	0.10	0.19	0.20	0.31	0.23
12	0.05	0.05	0.14	0.21	0.32	0.15
24	0.02	0.05	0.13	0.22	0.29	0.10
30	NM	0.04	0.11	0.16	0.29	0.11
48	NM	0.04	0.07	0.16	0.24	NM
72	NM	+	0.02	0.10	0.15	NM
96	NM	+	+	+	0.09	NM
120	NM	+	+	+	0.05	NM
144	NM	NM	NM	NM	+	NM
168	NM	NM	NM	NM	+	NM

NM Not measured

+ Below limit of detection (0.02 - 0.06  $\mu\text{g}/\text{ml}$ )

TABLE 11

Radioactivity in 30 second fractions of hplc eluate after injection  
 of extracts of plasma collected after oral administration of  
<sup>14</sup>C-WR 171,669.HCl at a dose level of 5 mg/kg  
 Results are expressed as % eluted radioactivity

Time (hours)	Fractions			
	6 - 9	14 - 15	17 - 19 <sup>†</sup>	23 - 26 <sup>*</sup>
1	+	+	+	100
2	17.6	+	5.3	77.1
3	30.3	+	5.8	63.9
4	37.6	+	10.3	52.1
5	33.3	+	11.9	54.8
6	33.6	+	16.0	50.5
7	33.6	+	18.4	48.0
12	48.5	+	25.0	26.5
24	88.5	+	11.5	+

+ Not detected, see Appendix 5 for limits of detection

\* Corresponds to WR 171,669

<sup>†</sup> Corresponds to WR 178,460

TABLE 12

Radioactivity in 30 second fractions of hplc eluate after injection  
 of extracts of plasma collected after oral administration of  
<sup>14</sup>C-WR 171,669.HCl at a dose level of 10 mg/kg  
 Results are expressed as % eluted radioactivity

Time (hours)	Fractions			
	5 - 12	14 - 15	17 - 19 <sup>†</sup>	23 - 26 <sup>*</sup>
1	9.3	+	+	90.7
2	39.6	+	5.1	55.3
3	49.2	+	7.8	43.0
4	49.2	+	12.8	38.1
5	53.6	+	14.8	31.7
6	46.8	+	15.7	37.5
7	44.0	+	22.5	33.5
12	74.6	4.4	8.7	12.3
24	59.6	+	16.7	23.6
30	65.6	4.2	6.7	23.6
48	70.0	8.5	8.3	13.1
72	94.7	+	+	5.3
96	100.0	+	+	+
120	100.0	+	+	+

+ Not detected, see Appendix 5 for limits of detection

\* Corresponds to WR 171,669

<sup>†</sup> Corresponds to WR 178,460

TABLE 13

Radioactivity in 30 second fractions of hplc eluate after injection  
 of extracts of plasma collected after oral administration of  
<sup>14</sup>C-WR 171,669.HCl at a dose level of 20 mg/kg  
 Results are expressed as % eluted radioactivity

Time (hours)	Fractions			
	6 - 10	14 - 15	17 - 19 <sup>+</sup>	23 - 26 <sup>*</sup>
0.5	6.6	+	+	93.4
1	21.4	+	+	78.6
2	14.1	+	7.6	78.2
3	22.2	+	9.4	68.4
4	26.1	+	12.2	61.7
5	33.3	+	13.2	53.5
6	33.3	1.5	14.6	50.6
7	46.0	3.1	20.6	30.3
12	50.3	2.0	17.1	30.6
24	55.3	4.9	24.6	15.2
30	55.8	11.3	24.4	8.5
48	75.0	9.0	16.0	+
72	82.2	11.6	6.2	+
96	77.5	10.0	+	12.5
120	100.0	+	+	+

+ Not detected, see Appendix 5 for limits of detection

\* Corresponds to WR 171,669

<sup>+</sup> Corresponds to WR 178,460

TABLE 14

Radioactivity in 30 second fractions of hplc eluate after injection  
 of extracts of plasma collected after oral administration of  
<sup>14</sup>C-WR 171,669.HCl at a dose level of 40 mg/kg  
 Results are expressed as % eluted radioactivity

Time (hours)	Fractions			
	5 - 10	14 - 15	17 - 19 <sup>+</sup>	22 - 26*
1	40.4	+	+	59.6
2	14.0	+	4.9	81.2
3	15.1	+	4.7	80.2
4	20.9	+	7.1	72.0
5	20.1	+	10.2	69.6
6	21.7	+	11.9	66.3
7	21.1	+	12.3	66.6
12	34.3	+	17.6	48.1
24	42.4	+	27.8	29.8
30	48.8	+	24.6	26.7
48	63.6	+	26.7	9.7
72	70.5	6.4	17.0	6.1
96	100.0	+	+	+
120	100.0	+	+	+

+ Not detected, see Appendix 5 for limits of detection

\* Corresponds to WR 171,669

<sup>+</sup> Corresponds to WR 178,460



TABLE 15

Radioactivity in 30 second fractions of hplc eluate after injection  
of extracts of plasma collected after oral administration of  
<sup>14</sup>C-WR 171,669.HCl at a dose level of 60 mg/kg  
Results are expressed as % eluted radioactivity

Time (hours)	Fractions			
	6 - 10	14 - 15	17 - 19 <sup>/</sup>	22 - 26 <sup>*</sup>
1	23.0	+	+	77.0
2	27.7	+	+	72.3
3	22.5	+	12.5	65.0
4	22.8	+	12.1 <sup>#</sup>	65.1 <sup>#</sup>
5	27.5	+	15.3	57.2
6	20.2	+	19.6	60.3
7	19.9	+	23.7	56.4
12	35.8	+	28.3	36.0
24	22.6	3.4	18.1	55.9
30	30.0	2.7	24.0	43.4
48	41.6	6.8	27.1	24.5
72	60.2	8.0	19.9	11.9
96	72.6	11.1	10.1	6.1
120	78.9	12.6	8.5	+
144	89.3	10.7	+	+
168	100.0	+	+	+

+ Not detected, see Appendix 5 for limits of detection

\* Corresponds to WR 171,669

<sup>/</sup> Corresponds to WR 178,460

<sup>#</sup> Fractions 19,20 and 26-28

TABLE 16

Radioactivity in 30 second fractions of hplc eluate after injection  
 of extracts of plasma collected after oral administration of  
<sup>14</sup>C-WR 171,669.HCl at a dose level of 100 mg/kg  
 Results are expressed as % eluted radioactivity

Time (hours)	Fractions			
	7 - 9	14 - 15	17 - 20 <sup>+</sup>	22 - 27 <sup>*</sup>
1	9.4	+	+	90.6
2	5.2	+	5.5	89.3
3	12.4	+	11.1	76.5
4	15.3	+	16.0	68.7
5	17.5	+	18.7	63.8
6	14.1	+	21.0	64.9
7	+	+	29.2	70.8
12	35.5	+	25.7	38.8
24	44.4	9.7	23.9	22.0
30	44.6	13.5	26.9	15.0

+ Not detected, see Appendix 5 for limits of detection

\* Corresponds to WR 171,669

<sup>+</sup> Corresponds to WR 178,460

TABLE 17

Observed (O) and expected (E) values for the fitted models for total radioactivity concentrations in plasma  
Results are expressed as dpm/ml

Time (hours)	Nominal dose level (mg/kg)											
	5		10		20		40		60		100	
	O	E	O	E	O	E	O	E	O	E	O	E
0.5	190	191	425	440	1616	1618	120	113	107	103	296	298
1	514	510	856	740	3701	3501	379	499	355	410	688	663
2	900	838	1055	1081	3473	3377	1134	1002	821	878	974	1005
3	891	937	1197	1234	3208	3168	1506	1280	1305	1202	1111	1106
4	862	931	1210	1299	2947	2978	2104	1430	1706	1424	1021	1118
5	857	883	1218	1322	2753	2805	2376	1507	1850	1576	1198	1096
6	875	822	1239	1325	2488	2648	2399	1543	1891	1678	1106	1063
7	844	761	1194	1319	2385	2505	2233	1555	1765	1745	1082	1026
12	533	555	1476	1257	2055	1957	1619	1505	1503	1833	800	861
24	392	417	824	1106	1338	1308	1061	1317	2188	1701	604	603
30	372	389	1604	1037	1130	1152	858	1231	1626	1626	542	524
48	315	325	1285	857	1132	908	830	1009	1196	1417	387	389
72	270	257	984	666	974	734	799	778	1022	1181	310	313
96	244	204	720	519	721	605	784	605	1242	984	272	273
120	179	161	493	407	548	500	615	475	843	822	235	245
144	121	127	320	320	398	413	389	377	750	686	225	221
168	100	101	218	253	304	341	302	303	631	574	194	200
192	78	80	174	202	229	282	250	247	518	481	187	181
240	48	50	124	133	149	192	165	172	310	340	144	148
288	ND	-	99	92	121	131	126	128	229	242	135	121
360	ND	-	62	60	91	74	94	92	144	150	96	90
432	ND	-	ND	-	63	42	72	72	104	97	62	67
504	ND	-	ND	-	ND	-	57	60	66	67	ND	-
576	ND	-	ND	-	ND	-	55	52	49	49	ND	-

ND Not detected

TABLE 18

Parameter estimates for the fitted models for total  
radioactivity concentrations in plasma

Parameter (units)	Nominal dose level (mg/kg)					
	5	10	20	40	60	100
$\tau$ (h)	0.28	0	0.40	0.38	0.35	0.22
$\lambda_1$ ( $h^{-1}$ )	0.655	0.752	5.99	0.566	0.355	0.959
$\lambda_2$ ( $h^{-1}$ )	0.233	0.0110	0.0994	0.0122	0.0078	0.0575
$\lambda_3$ ( $h^{-1}$ )	0.0099	0	0.0080	0.0016	0	0.0042
$A_2$ (dpm/ml)	1433	1397	2461	1593	2013	943
$A_3$ (dpm/ml)	519	33	1294	128	26	401
$h_1$ (h)	1.06	0.92	0.12	1.22	1.95	0.72
$h_2$ (h)	2.97	63.1	6.97	56.7	89.3	12.1
$h_3$ (h)	70.1	-	87.1	433	-	167

TABLE 19

Fitted\* models for concentrations of WR 171,669 free base in plasma (O = Observed, E = Expected)  
Results are expressed as µg/ml

Time (hours)	Nominal dose level (mg/kg)											
	5		10		20		40		60		100	
	O	E	O	E	O	E	O	E	O	E	O	E
0.5	-	-	-	-	-	-	-	-	-	-	-	-
1	0.23	0.23	0.34	0.22	0.66	0.66	0.06	0.06	0.07	0.07	0.53	0.53
2	0.30	0.28	0.25	0.21	1.19	1.26	0.77	0.76	0.50	0.54	0.73	0.70
3	0.25	0.26	0.22	0.21	0.96	0.91	1.01	1.11	0.71	0.74	0.71	0.71
4	0.20	0.23	0.20	0.20	0.80	0.78	1.27	1.25	0.93	0.82	0.58	0.67
5	0.21	0.20	0.17	0.19	0.65	0.66	1.39	1.29	0.89	0.85	0.64	0.62
6	0.19	0.17	0.20	0.19	0.55	0.56	1.33	1.26	0.95	0.84	0.60	0.57
7	0.18	0.15	0.17	0.18	0.32	0.48	1.25	1.20	0.83	0.83	0.64	0.52
12	0.06	0.07	0.08	0.15	0.28	0.21	0.72	0.83	0.45	0.73	0.26	0.32
24	<0.03	0.01	0.08	0.10	0.09	0.03	0.27	0.30	1.02	0.52	0.11	0.11
30	-	-	0.17	0.08	0.04	0.01	0.19	0.18	0.59	0.44	0.07	0.06
48	-	-	0.07	0.04	<0.02	0.00	0.07	0.04	0.25	0.26	-	-
72	-	-	<0.03	0.02	<0.03	0.00	<0.05	0.00	0.10	0.13	-	-
96	-	-	<0.03	0.01	<0.06	0.00	<0.05	0.00	0.06	0.07	-	-
120	-	-	<0.04	0.00	<0.06	0.00	<0.05	0.00	<0.06	0.03	-	-
144	-	-	-	-	-	-	-	-	<0.06	0.02	-	-
168	-	-	-	-	-	-	-	-	<0.07	0.01	-	-
Model parameters												
$\tau$	0		0		0.4282		0.9396		0.9045		0.0946	
$\lambda_1$	1.128		29.0		9.2978		0.5507		0.7690		1.0732	
$\lambda_2$	0.1531		0.0354		0.1624		0.0860		0.0282		0.0937	
A	0.4388		0.2303		1.3887		2.1509		0.9979		0.9897	
$h_1$	$0.614 \pm 0.201$		$0.024 \pm 0.013$		$0.075 \pm 0.064$		$1.259 \pm 0.191$		$0.901 \pm 0.408$		$0.646 \pm 0.500$	
$h_2$	$4.528 \pm 0.873$		$19.57 \pm 9.64$		$4.268 \pm 0.790$		$8.062 \pm 0.704$		$24.59 \pm 4.112$		$7.399 \pm 0.943$	
R	0.9487		0.5655		0.9622		0.9900		0.8367		0.9739	

# Data were fitted to the equation:  $c = A(e^{-\lambda_2(t-\tau)} - e^{-\lambda_1(t-\tau)})$

TABLE 20

Weighted residual mean squares for the fitted curves  
(weights were scaled to have an average value of unity)

Nominal dose level (mg/kg)	Unchanged compound	Total radioactivity
5	0.00065	133.7
10	0.00354	1917.4
20	0.00513	1182.0
40	0.00194	1153.9
60	0.01255	484.0
100	0.00256	120.6

TABLE 21

Extraction\* of radioactivity from faeces samples up to 72 hours  
after single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl to dogs at various levels  
Results are expressed as % faecal radioactivity

Nominal dose level (mg/kg)	Time period (hours)	Extract			Total*
		1	2	3	
5	0-24	15.2	26.9	22.4	64.5 (28.2)
	24-48	16.0	25.1	15.6	56.7 ( 8.8)
	48-72	67.5	10.6	5.1	83.2 (11.0)
10	0-24	59.2	24.3	8.9	92.4 (21.1)
	24-48	29.2	27.9	14.9	72.0 (17.9)
	48-72	6.6	28.3	18.7	53.6 ( 6.4)
20	0-24	42.2	26.8	14.9	83.9 (46.4)
	24-48	28.7	29.9	11.6	70.2 ( 8.5)
	48-72	18.0	29.1	16.5	63.6 ( 4.6)
40	0-24	41.8	31.2	15.7	88.7 (49.2)
	24-48	13.3	24.2	27.9	65.4 (10.0)
	48-72	23.1	33.5	17.3	73.9 ( 2.8)
60	0-24	22.2	24.7	26.0	72.9 (43.7)
	24-48	12.9	30.7	19.6	63.2 ( 4.5)
100	0-24	9.6	13.6	29.5	52.7 (44.4)
	24-48	22.2	25.7	19.2	67.1 ( 1.1)
	48-72	15.9	28.4	15.9	60.2 ( 0.5)

\* Figures in brackets are total extracted expressed as % dose

# Extract 1, methanol; Extracts 2,3, methanol : diethylamine (9:1, v/v)

TABLE 22

Radioactivity in 30 second fractions of hplc eluate after  
 injection of extracts of faeces collected up to 72 hours  
 after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl  
 to a beagle dog at a nominal dose level of 5 mg/kg

Time interval (hours)	Zone No. #	Fraction numbers (inclusive)	% eluted radioactivity	% dose
0-24 (extract 1)	1	4 - 11	32.1	2.1
	2	22 - 25	11.4	0.8
	3	35 - 39	55.4	3.7
	Others	16	1.2	0.08
0-24 (extracts 2&3)	1	4 - 15	32.6	7.0
	2	18 - 21	11.4	2.5
	3	27 - 31	55.9	12.0
24-48	1	4 - 10	79.6	7.0
	2	22 - 23	15.7	1.4
	3	33	4.7	0.4
48-72	1	4 - 11	76.0	8.4
	2	19 - 22	15.0	1.7
	3	31 - 33	9.0	1.0

\* Zone 3 corresponds to WR 171,669



TABLE 23

Radioactivity in 30 second fractions of hplc eluate after  
 injection of extracts of faeces collected up to 72 hours  
 after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl  
 to a beagle dog at a nominal dose level of 10 mg/kg

Time interval (hours)	Zone No. #	Fraction numbers (inclusive)	% eluted radioactivity	% dose
0-24 (extract 1)	1	4 - 18	7.9	1.1
	2	22 - 27	3.4	0.5
	3	33 - 43	87.8	11.9
	Others	29, 30, 46-48	0.9	0.1
0-24 (extracts 2&3)	1	5 - 12	13.3	1.0
	2	19 - 20	3.3	0.3
	3	27 - 31	82.3	6.3
	Others	14, 23	1.2	0.09
24-48	1	4 - 18	39.5	7.1
	2	20 - 24	9.7	1.7
	3	31 - 36	50.8	9.1
48-72	1	4 - 11	77.0	4.9
	2	19 - 22	11.2	0.7
	3	30 - 32	10.0	0.6
	Others	16	1.8	0.1

\* Zone 3 corresponds to WR 171,669

TABLE 24

Radioactivity in 30 second fractions of hplc eluate after  
 injection of extracts of faeces collected up to 72 hours  
 after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl  
 to a beagle dog at a nominal dose level of 20 mg/kg

Time interval (hours)	Zone No. #	Fraction numbers (inclusive)	% eluted radioactivity	% dose
0-24 (extract 1)	1	4 - 21	13.1	3.1
	2	22 - 27	4.5	1.0
	3	32 - 42	81.3	18.9
	Others	28-30, 45-46	1.1	0.3
0-24 (extracts 2&3)	1	5 - 18	15.3	3.5
	2	19 - 24	4.7	1.1
	3	26 - 35	80.0	18.5
24-48	1	5 - 16	80.1	6.8
	2	19 - 22	11.0	0.9
	3	30 - 32	9.0	0.8
48-72	1	4 - 11	85.3	3.9
	2	20 - 22	6.4	0.3
	3	31 - 32	5.6	0.3
	Others	15 - 16	2.8	0.1

\* Zone 3 corresponds to WR 171,669

TABLE 25

Radioactivity in 30 second fractions of hplc eluate after  
 injection of extracts of faeces collected up to 72 hours  
 after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl  
 to a beagle dog at a nominal dose level of 40 mg/kg

Time interval (hours)	Zone No. #	Fraction numbers (inclusive)	% eluted radioactivity	% dose
0-24 (extract 1)	1	5 - 14	9.5	2.2
	2	18 - 25	4.6	1.1
	3	27 - 34	85.8	19.9
0-24 (extracts 2&3)	1	4 - 14	5.0	1.3
	2	17 - 24	3.8	1.0
	3	25 - 34	91.1	23.7
24-48	1	4 - 11	87.0	8.7
	2	16 - 18	10.8	1.1
	3	23 - 26	2.2	0.2
48-72	1	4 - 9	88.1	2.5
	2	17 - 19	11.9	0.3

\* Zone 3 corresponds to WR 171,669

TABLE 26

Radioactivity in 30 second fractions of hplc eluate after  
 injection of extracts of faeces collected up to 48 hours  
 after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl  
 to a beagle dog at a nominal dose level of 60 mg/kg

Time interval (hours)	Zone No. #	Fraction numbers (inclusive)	% eluted radioactivity	% dose
0-24 (extract 1)	1	6 - 11	5.5	0.7
	2	18 - 21	2.9	0.4
	3	27 - 34	90.4	12.0
	Others	14,25,26	1.2	0.2
0-24 (extracts 2&3)	1	4 - 11	4.8	1.5
	2	17 - 20	2.7	0.8
	3	25 - 31	92.0	28.0
	Others	22	0.5	0.2
24-48	1	4 - 11	49.7	2.2
	2	16 - 18	15.9	0.7
	3	23 - 26	33.3	1.5
48-72	No faeces sample for this time period			

\* Zone 3 corresponds to WR 171,669

TABLE 27

Radioactivity in 30 second fractions of hplc eluate after  
 injection of extracts of faeces collected up to 72 hours  
 after oral administration of  $^{14}\text{C}$ -WR 171,669.HCl  
 to a beagle dog at a nominal dose level of 100 mg/kg

Time interval (hours)	Zone No. *	Fraction numbers (inclusive)	% eluted radioactivity	% dose
0-24 (extract 1)	1	5 - 15	3.2	0.3
	2	19 - 22	2.1	0.2
	3	27 - 36	92.8	7.5
	Others	18, 23-26	1.8	0.1
0-24 (extracts 2&3)	1	4 - 13	2.6	0.9
	2	16 - 20	2.7	1.0
	3	25 - 33	93.7	34.0
	Others	21 - 24	1.0	0.4
24-48	1	5 - 10	67.4	0.7
	2	17 - 18	15.0	0.2
	3	24 - 25	17.6	0.2
48-72	1	5 - 8	88.3	0.4
	2	17	11.7	0.06

\* Zone 3 corresponds to WR 171,669

TABLE 28

Proportions of radioactivity in extracts of faeces  
associated with WR 171,669 after single oral doses of  
<sup>14</sup>C-WR 171,669.HCl to dogs at various levels

## (1) Results expressed as % extracted radioactivity

Time period (hours)	Nominal dose level (mg/kg)					
	5	10	20	40	60	100
0 - 24 (extract 1)	55.4	87.8	81.3	85.8	90.4	92.8
0 - 24 (extracts 2,3)	55.9	82.3	80.0	91.1	92.0	93.7
24 - 48 <sup>+</sup>	4.7	50.8	9.0	2.2	33.3	17.6
48 - 72 <sup>+</sup>	9.0	10.0	5.6	ND	NS	ND

## (2) Results expressed as % dose

Time period (hours)	Nominal dose level (mg/kg)					
	5	10	20	40	60	100
0 - 24 <sup>+</sup>	15.7	18.2	37.4	43.6	40.0	41.5
24 - 48 <sup>+</sup>	0.4	9.1	0.8	0.2	1.5	0.2
48 - 72 <sup>+</sup>	1.0	0.6	0.3	ND	NS	ND
Total 0 - 72	17.1	27.9	38.5	43.8	41.5	41.7

ND Not detected

NS No sample

<sup>+</sup> All 3 extracts for each time period

FIGURE 1

Cumulative excretion of radioactivity in the faeces of dogs after single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl

Key:  $\Delta$  5 mg/kg,  $\circ$  20 mg/kg,  $\square$  60 mg/kg  
 $\blacktriangle$  10 mg/kg,  $\bullet$  40 mg/kg,  $\blacksquare$  100 mg/kg

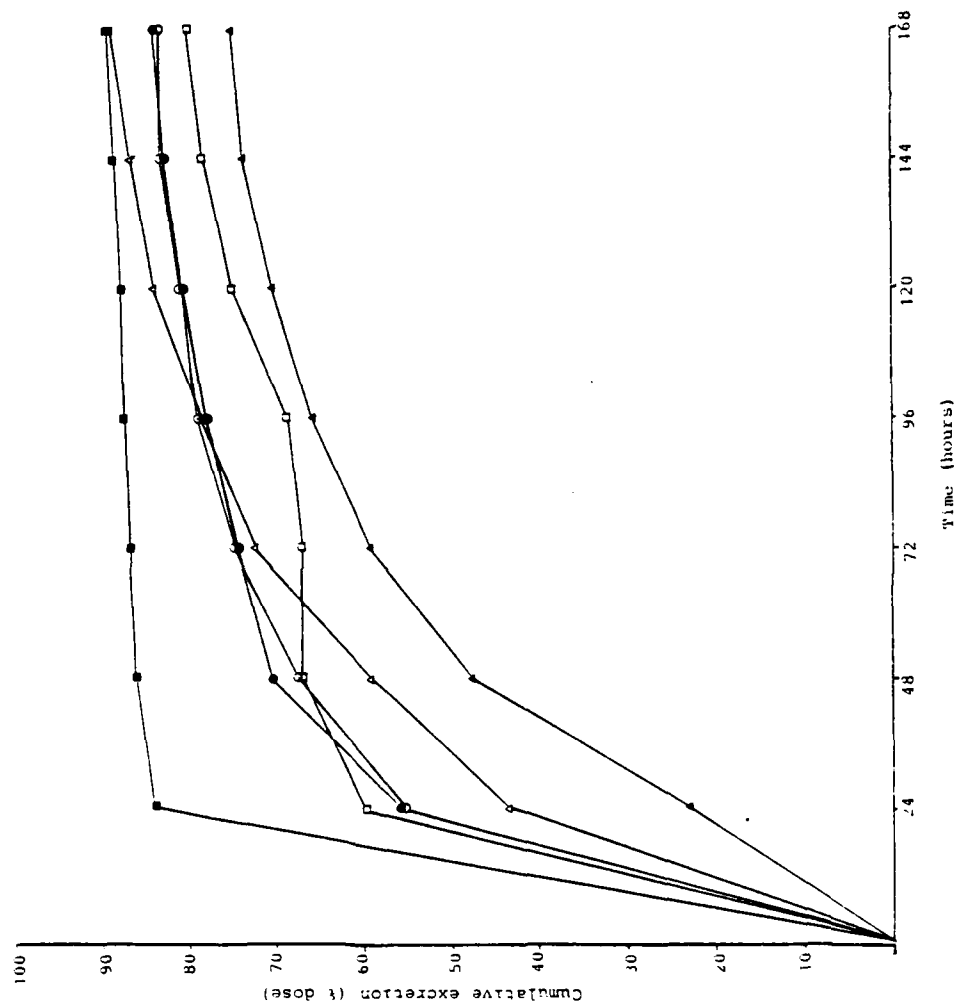


FIGURE 2

Concentrations of radioactivity in the plasma of dogs  
up to 72 hours after single oral doses  
of  $^{14}\text{C}$ -WR 171,669.HCl

Key:  $\Delta$  5 mg/kg,  $\circ$  20 mg/kg,  $\square$  60 mg/kg  
 $\blacktriangle$  10 mg/kg,  $\bullet$  40 mg/kg,  $\blacksquare$  100 mg/kg

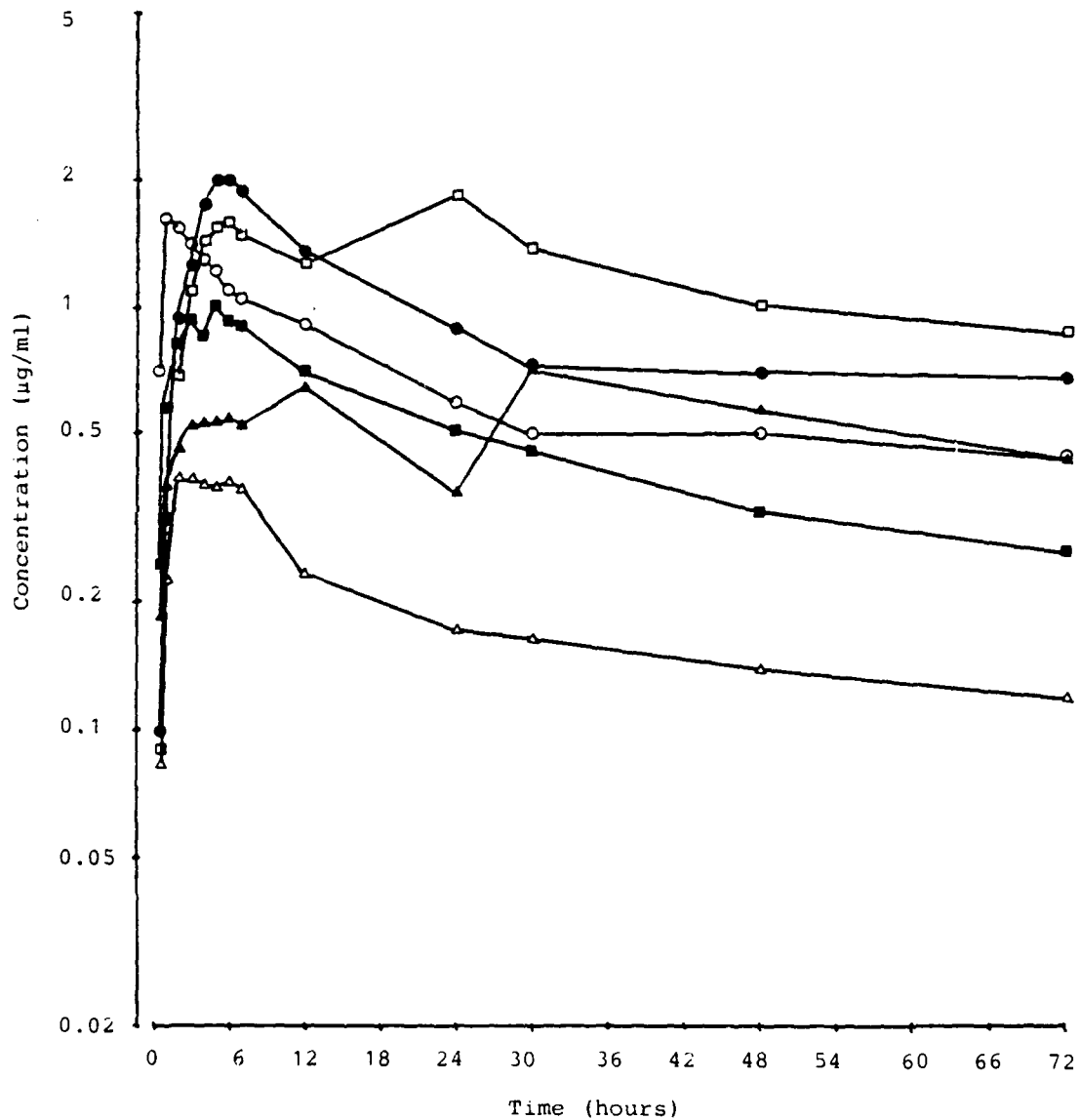




FIGURE 3

Concentrations of radioactivity in the plasma of dogs  
after single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl  
(Values before 6 hours not shown)

Key:  $\Delta$  5 mg/kg,  $\circ$  20 mg/kg,  $\square$  60 mg/kg  
 $\blacktriangle$  10 mg/kg,  $\bullet$  40 mg/kg,  $\blacksquare$  100 mg/kg

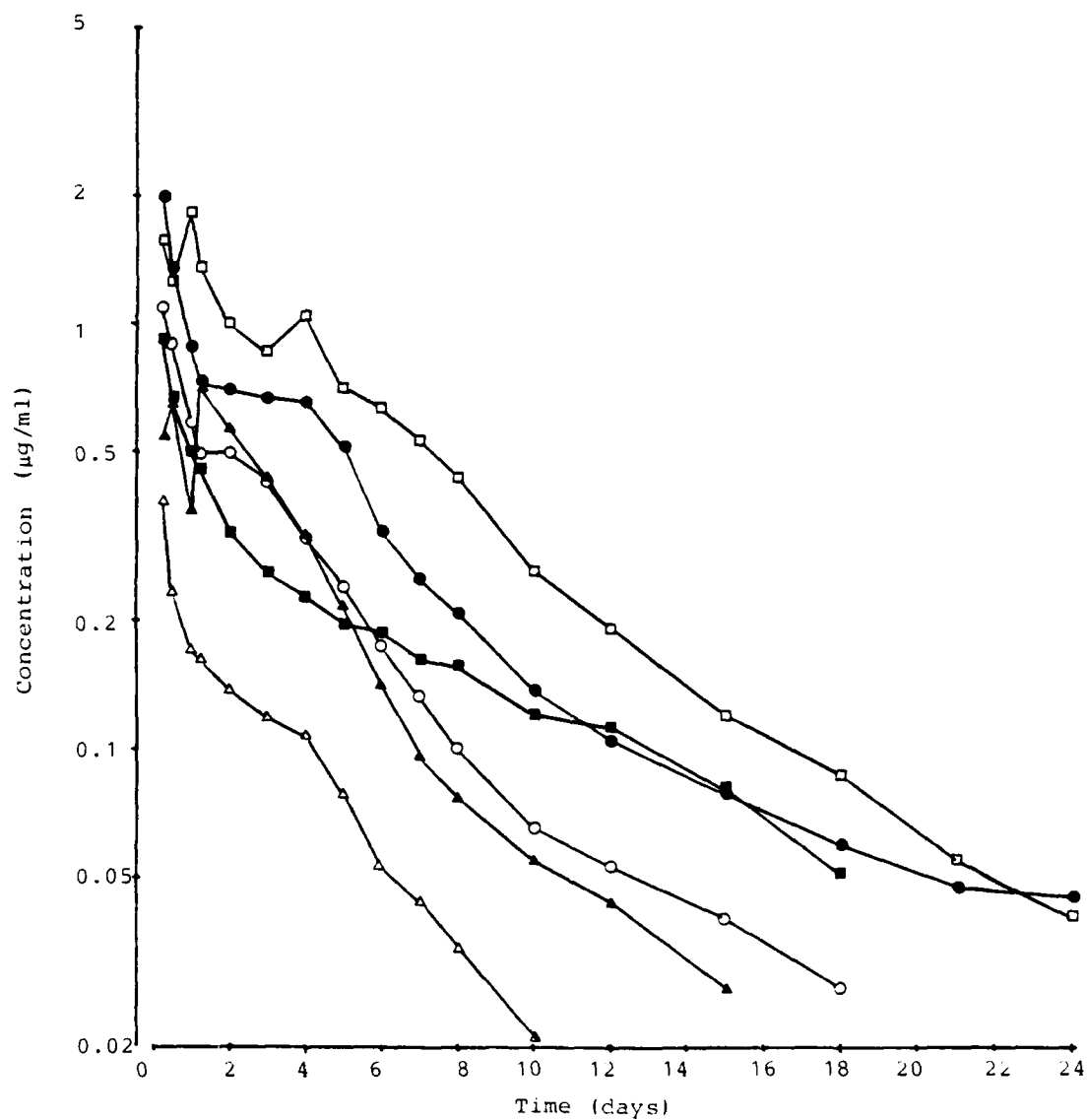


FIGURE 4

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 5 mg/kg

KEY: ▲-PLASMA      ◇-BLOOD

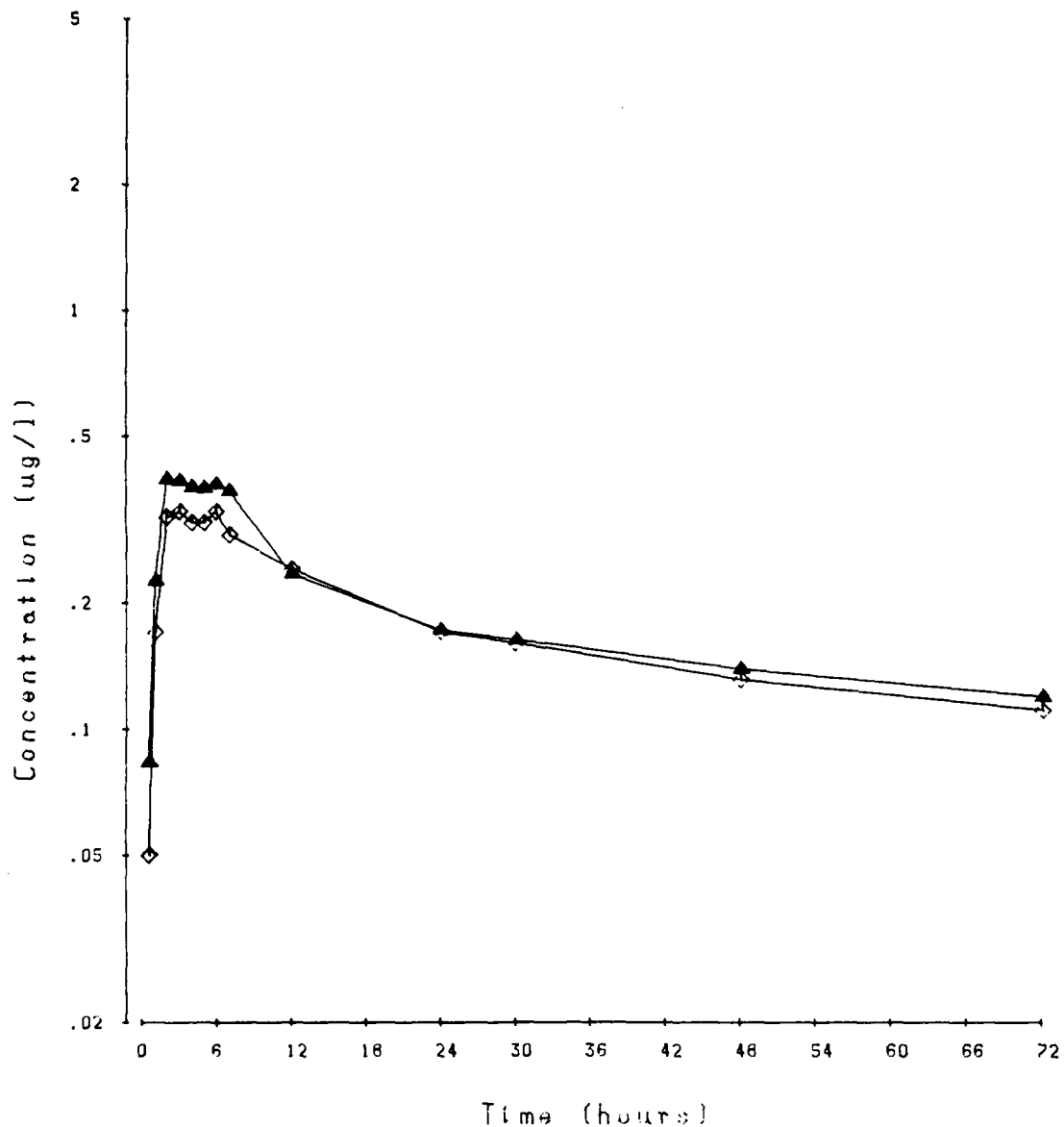


FIGURE 5

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 5 mg/kg (Values before 6 hours not shown)

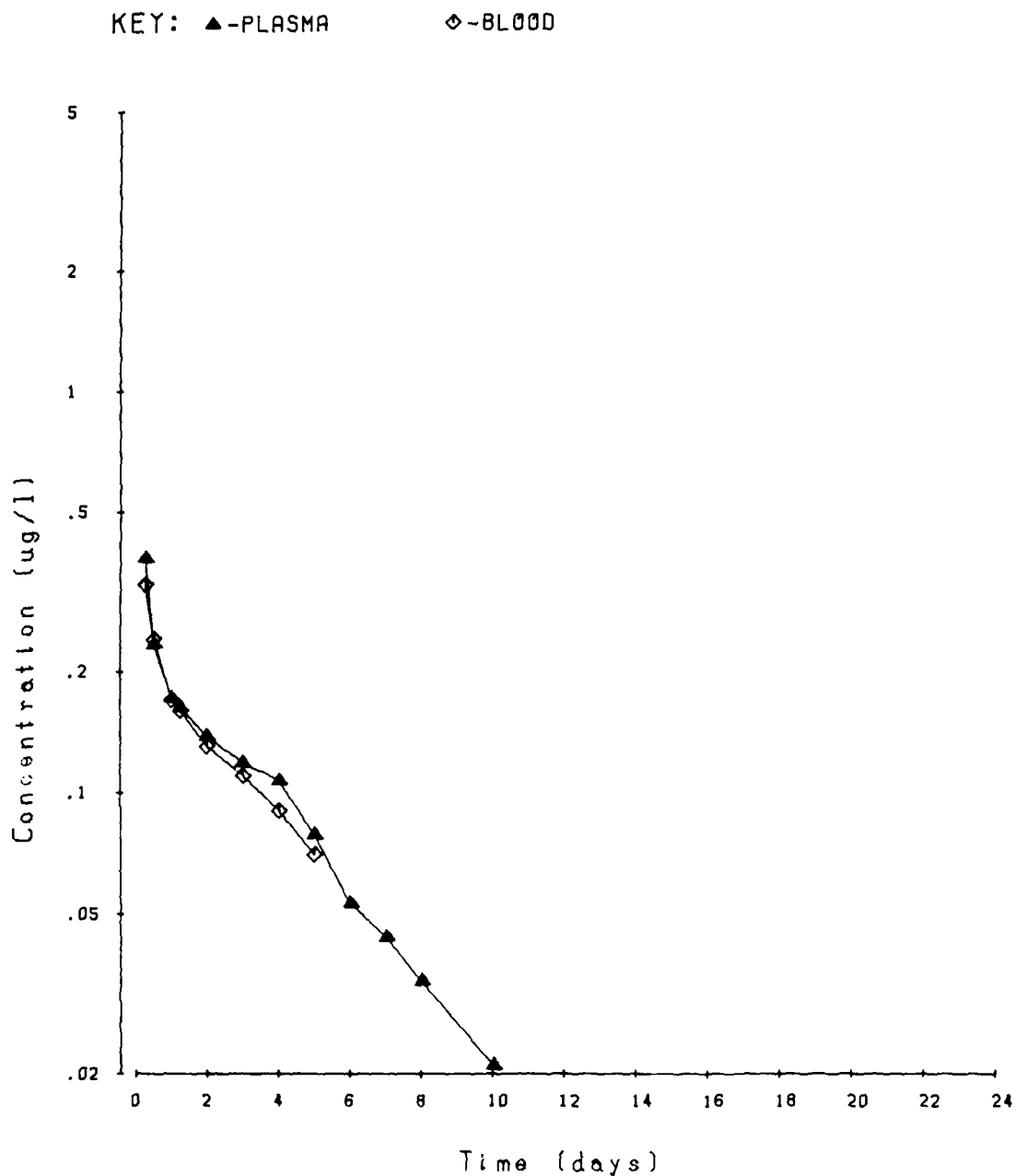


FIGURE 6

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 10 mg/kg

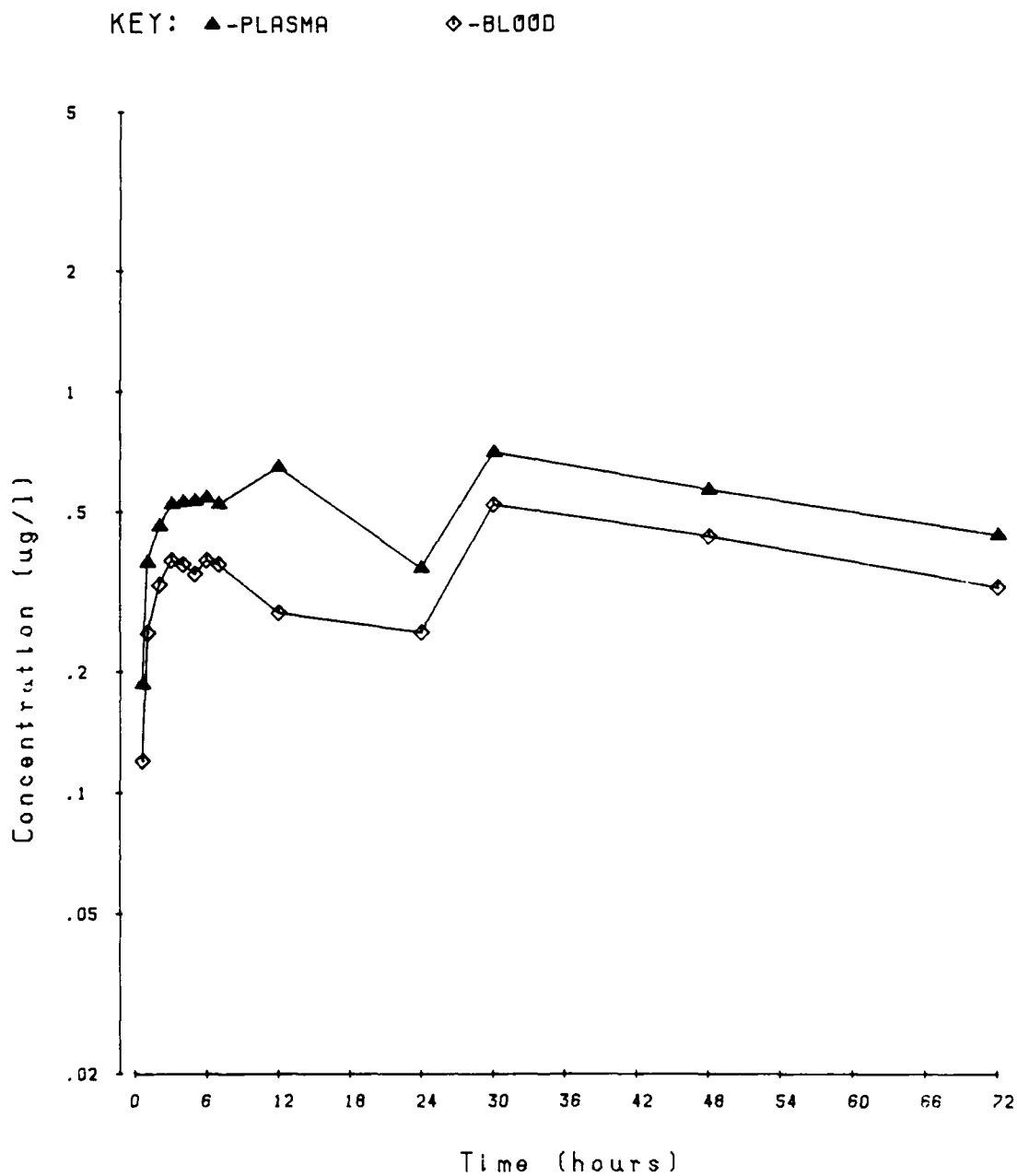


FIGURE 7

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 10 mg/kg (Values before 6 hours not shown)

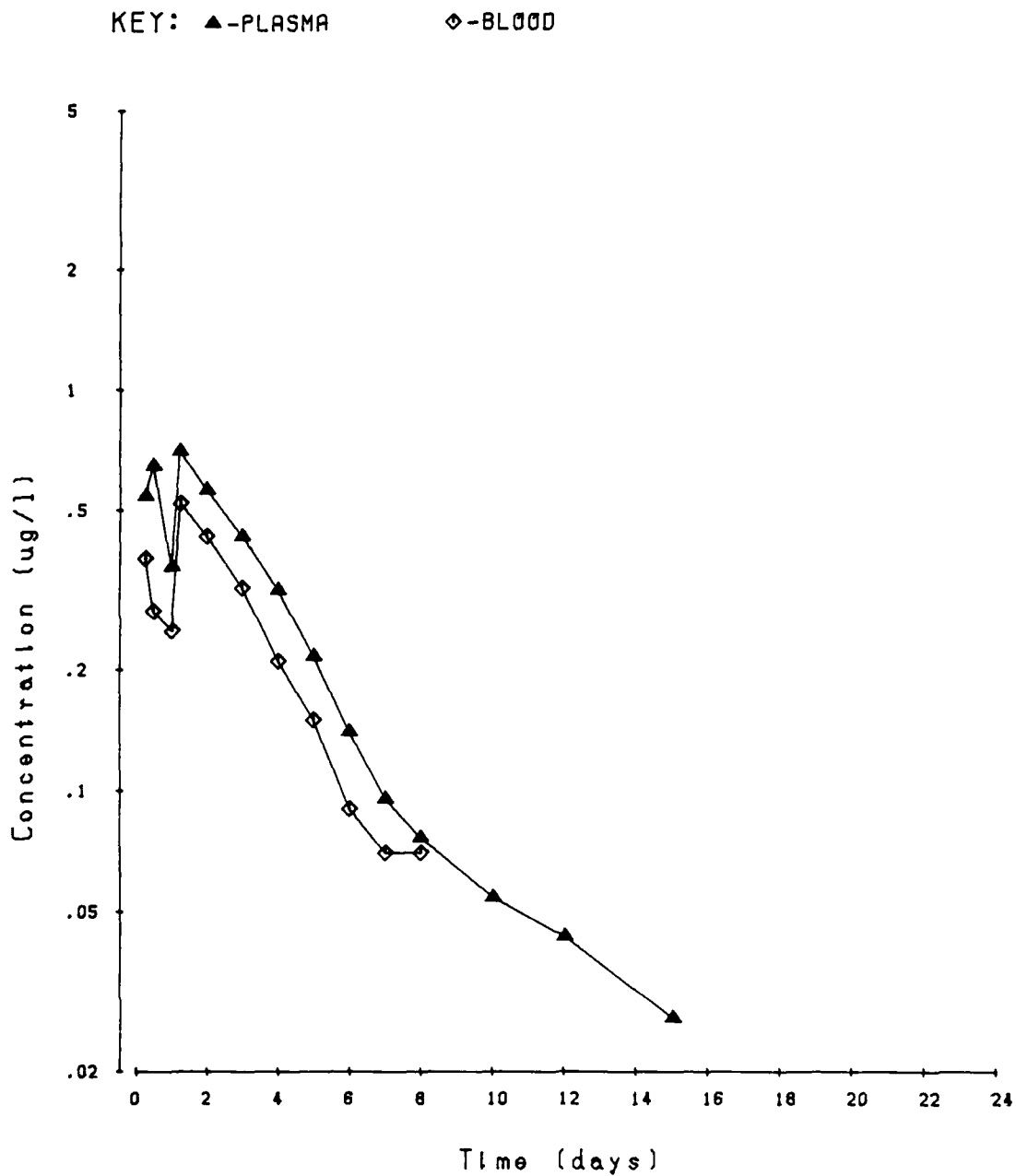


FIGURE 8

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 20 mg/kg

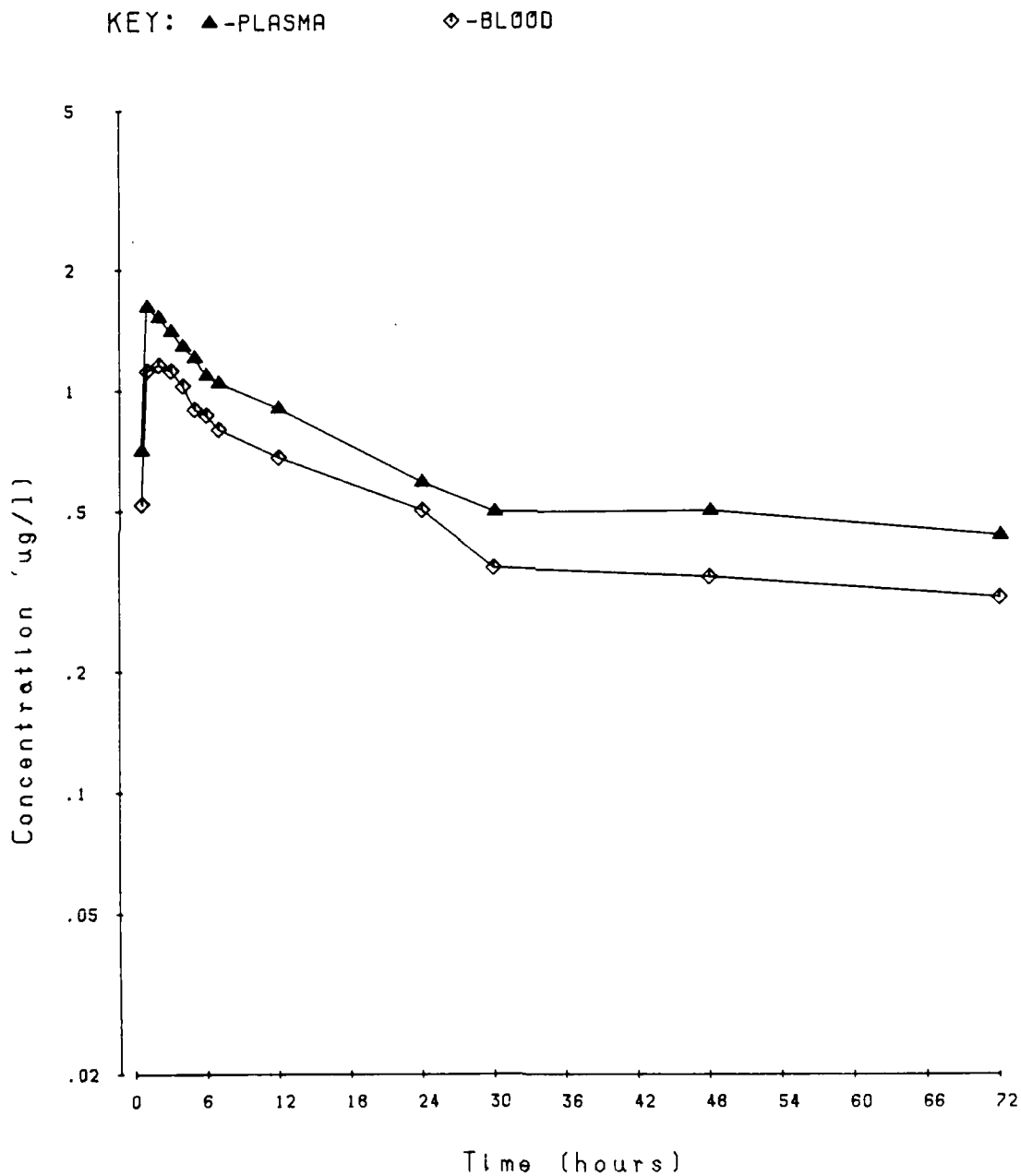


FIGURE 9

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 20 mg/kg (Values before 6 hours not shown)

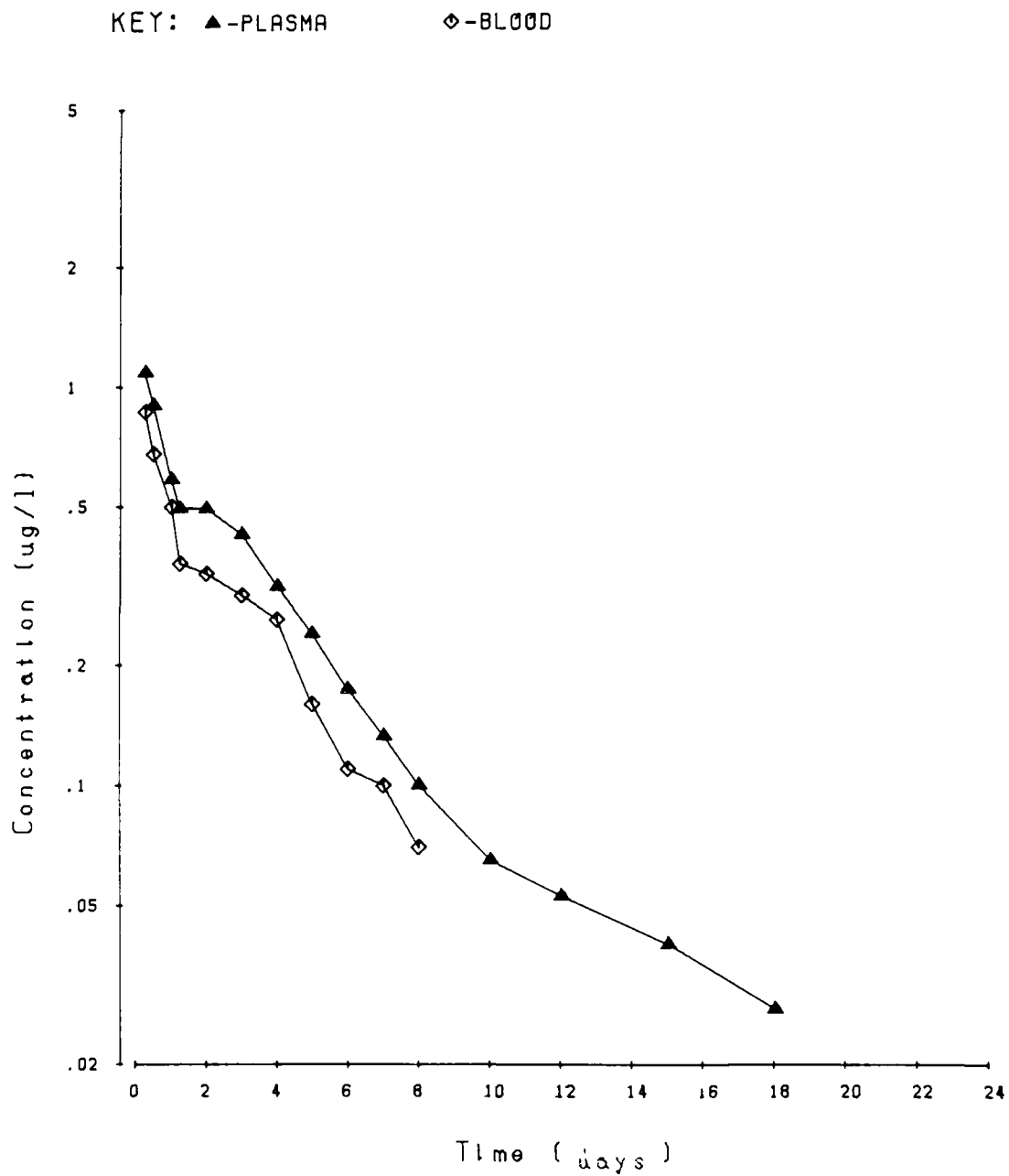


FIGURE 10

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 40 mg/kg

KEY: ▲-PLASMA      ◇-BLOOD

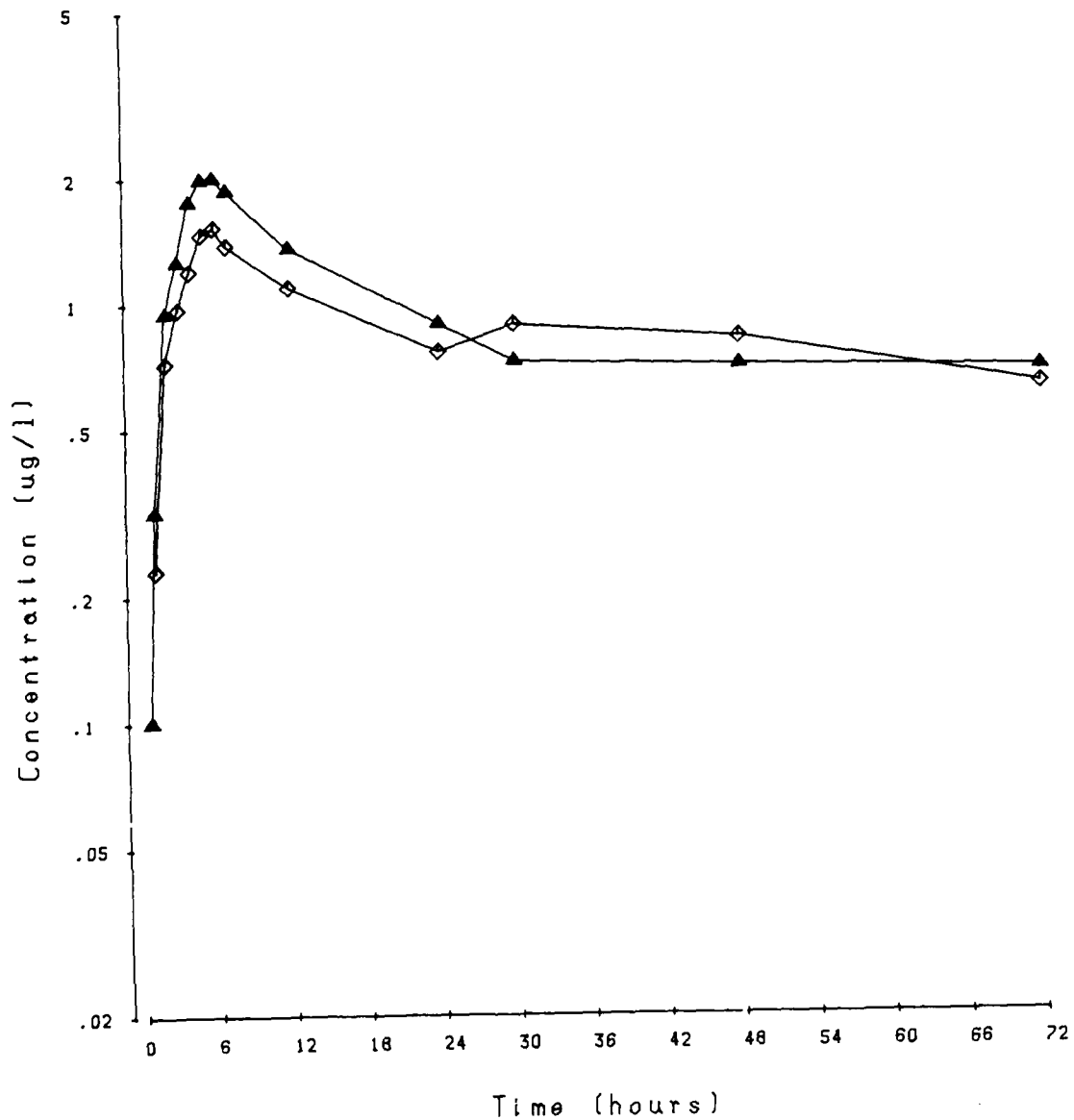




FIGURE 11

Concentrations of radioactivity in the plasma and whole-blood of a dog  
after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 40 mg/kg  
(Values before 6 hours not shown)

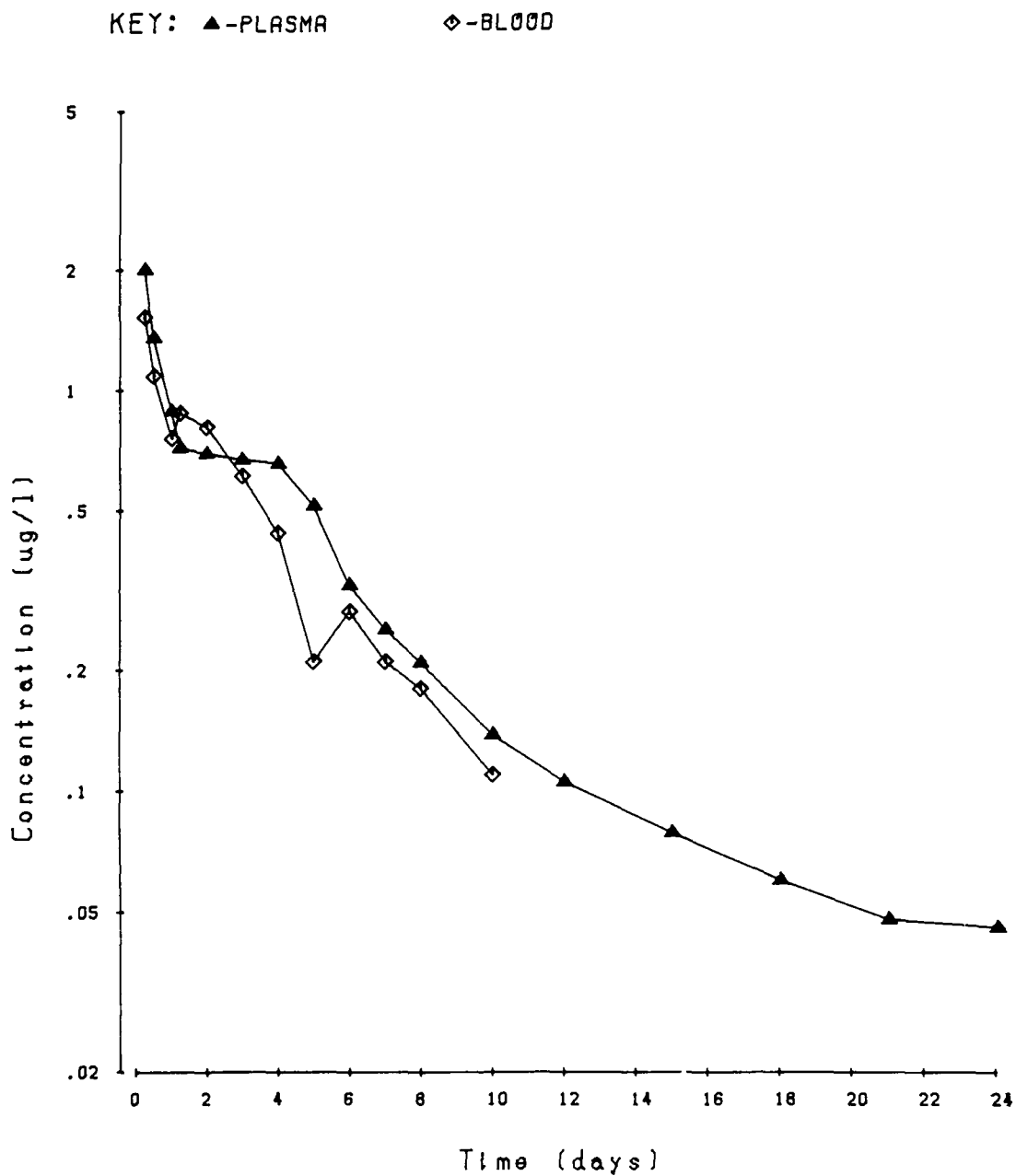


FIGURE 12

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 60 mg/kg

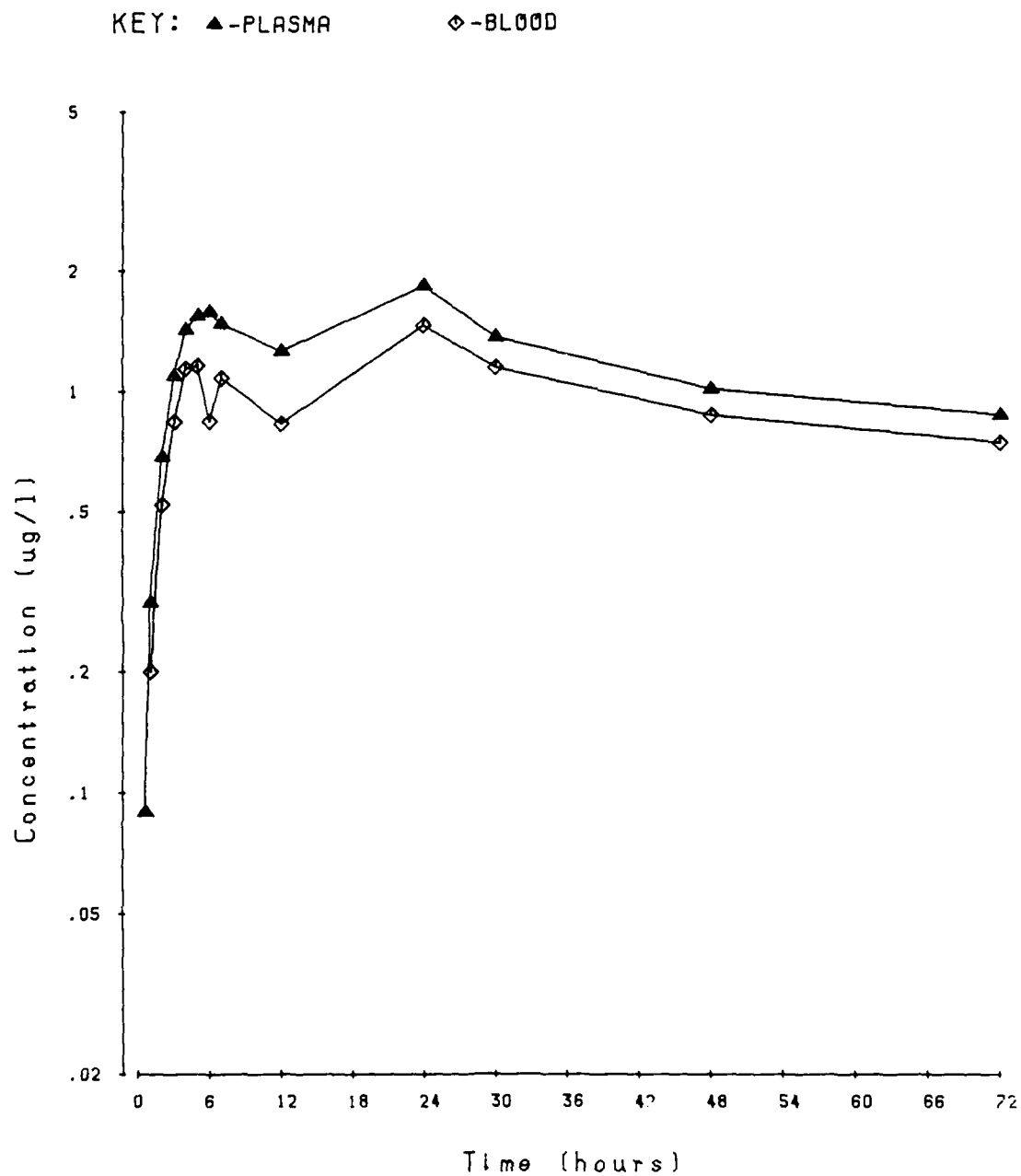


FIGURE 13

Concentrations of radioactivity in the plasma and whole-blood of a dog  
after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 60 mg/kg  
(Values before 6 hours not shown)

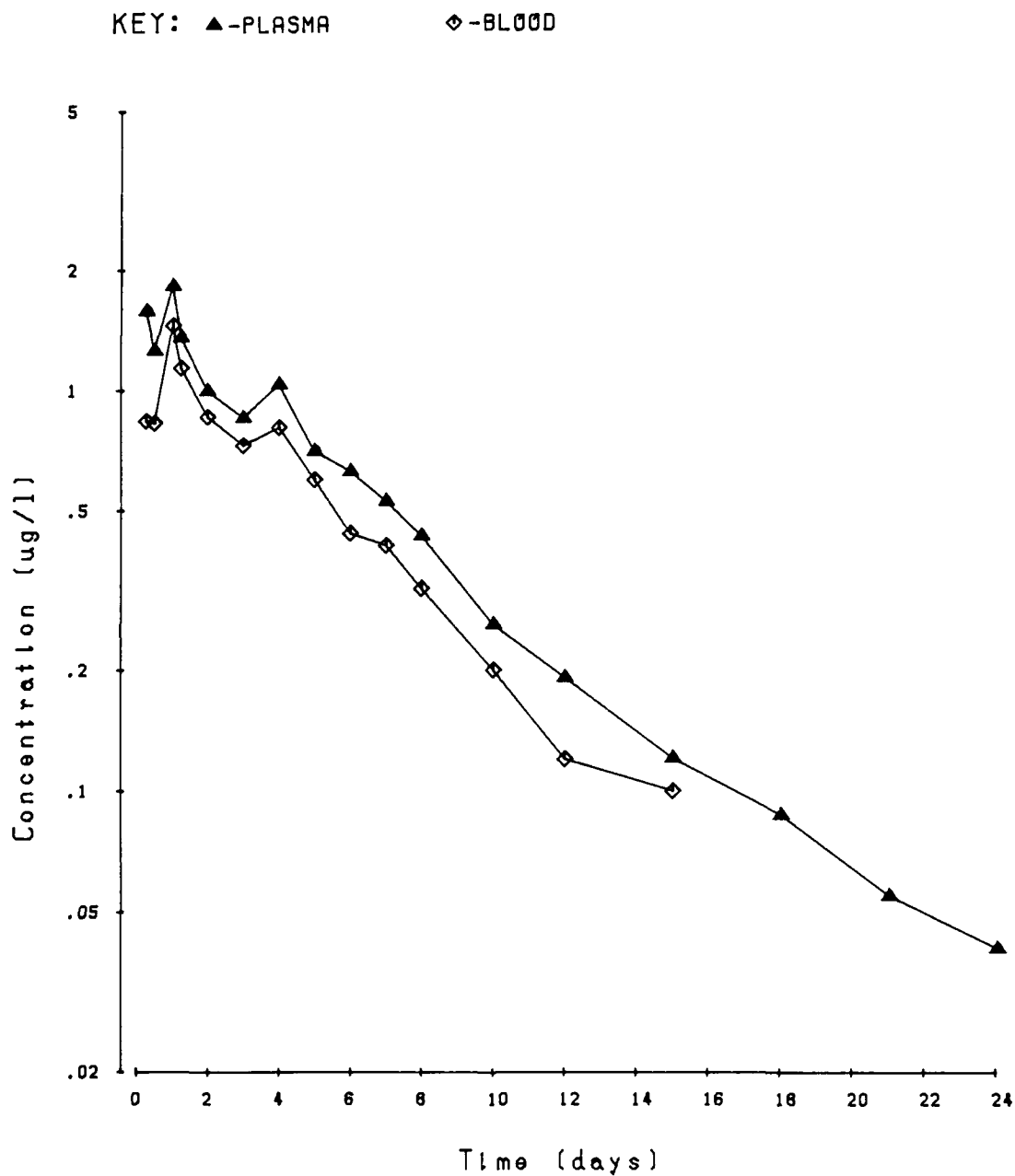


FIGURE 14

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 100 mg/kg

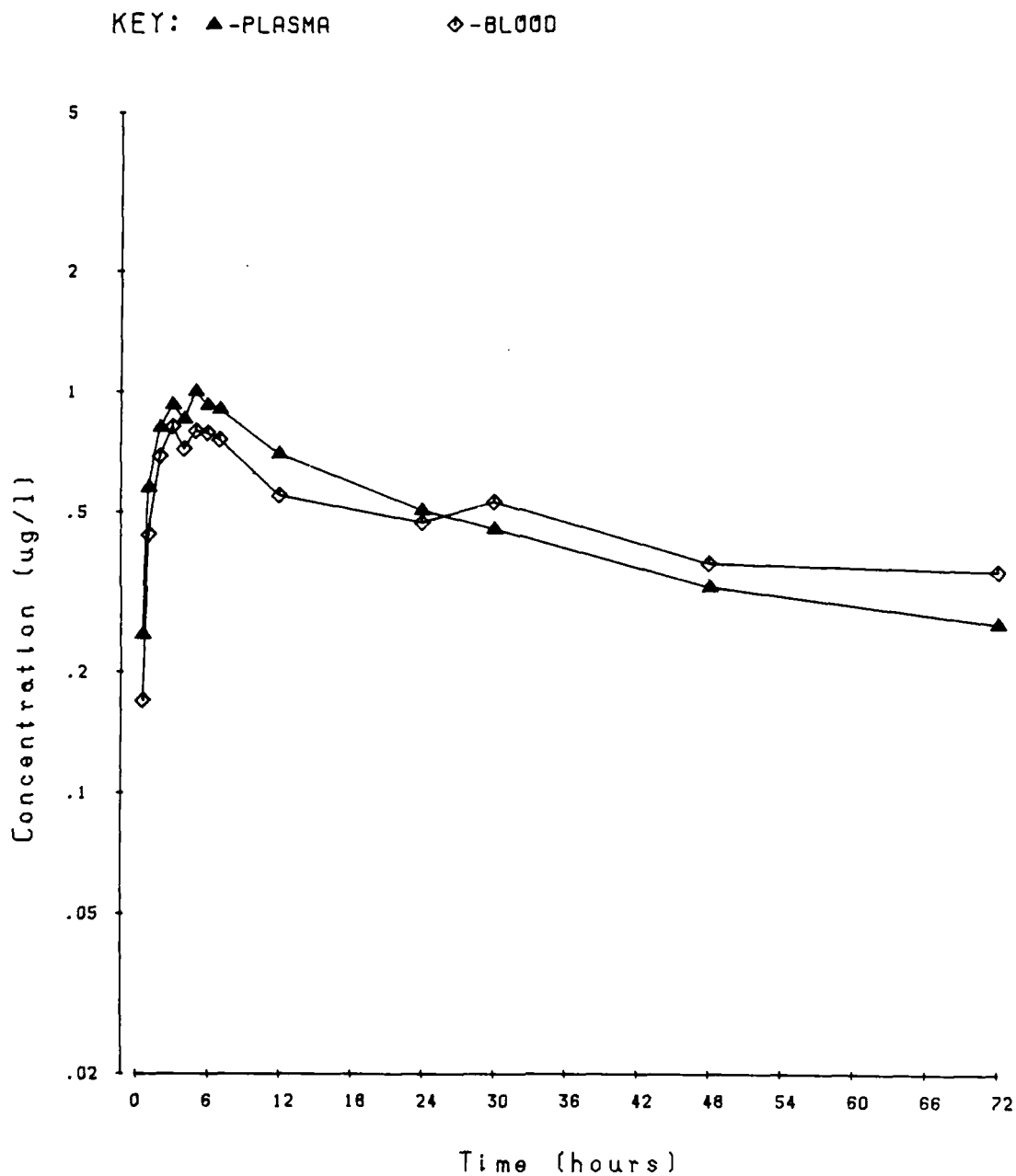


FIGURE 15

Concentrations of radioactivity in the plasma and whole-blood of a dog after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 100 mg/kg (Values before 6 hours not shown)

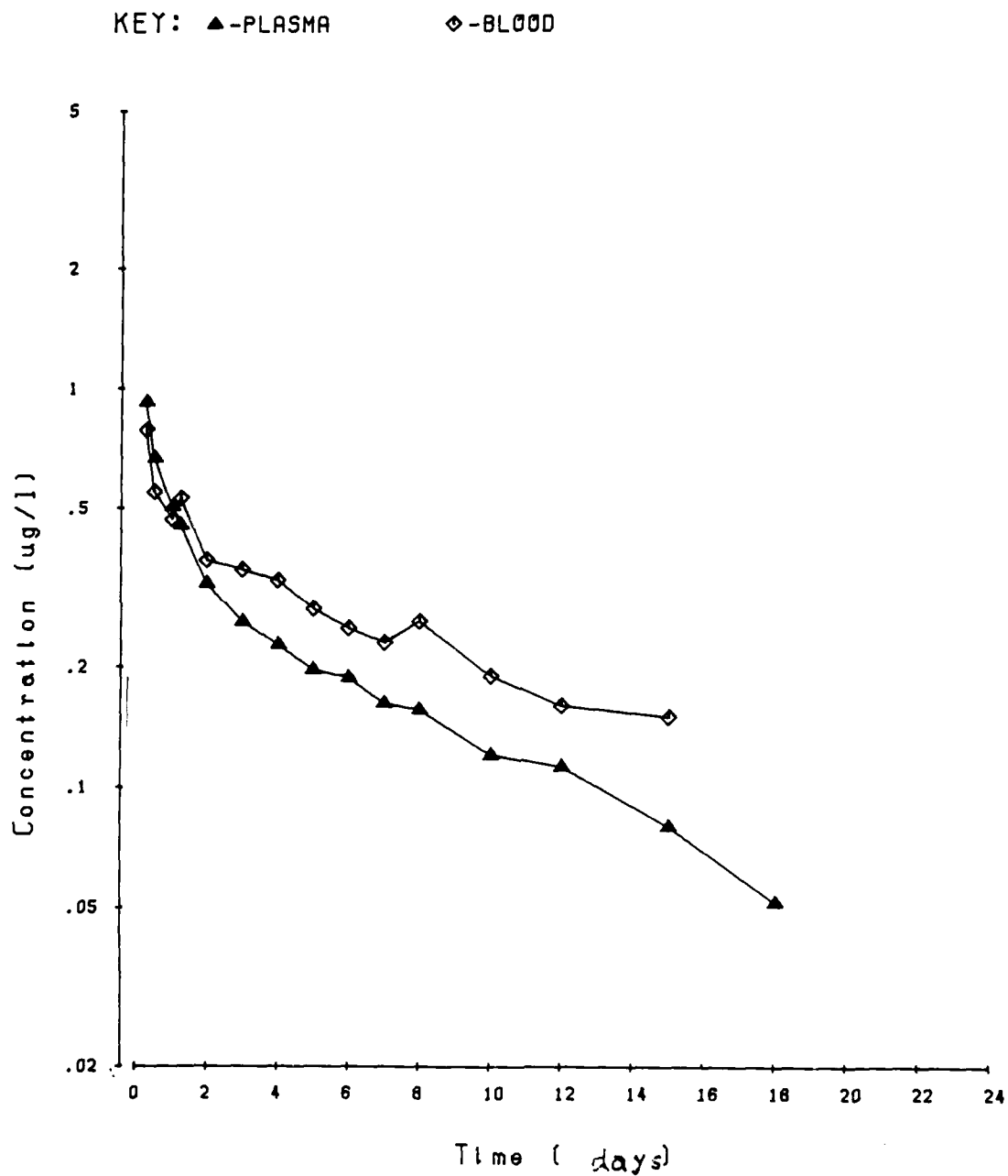


FIGURE 16

Concentrations of WR 171,669 free base in plasma after  
single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl

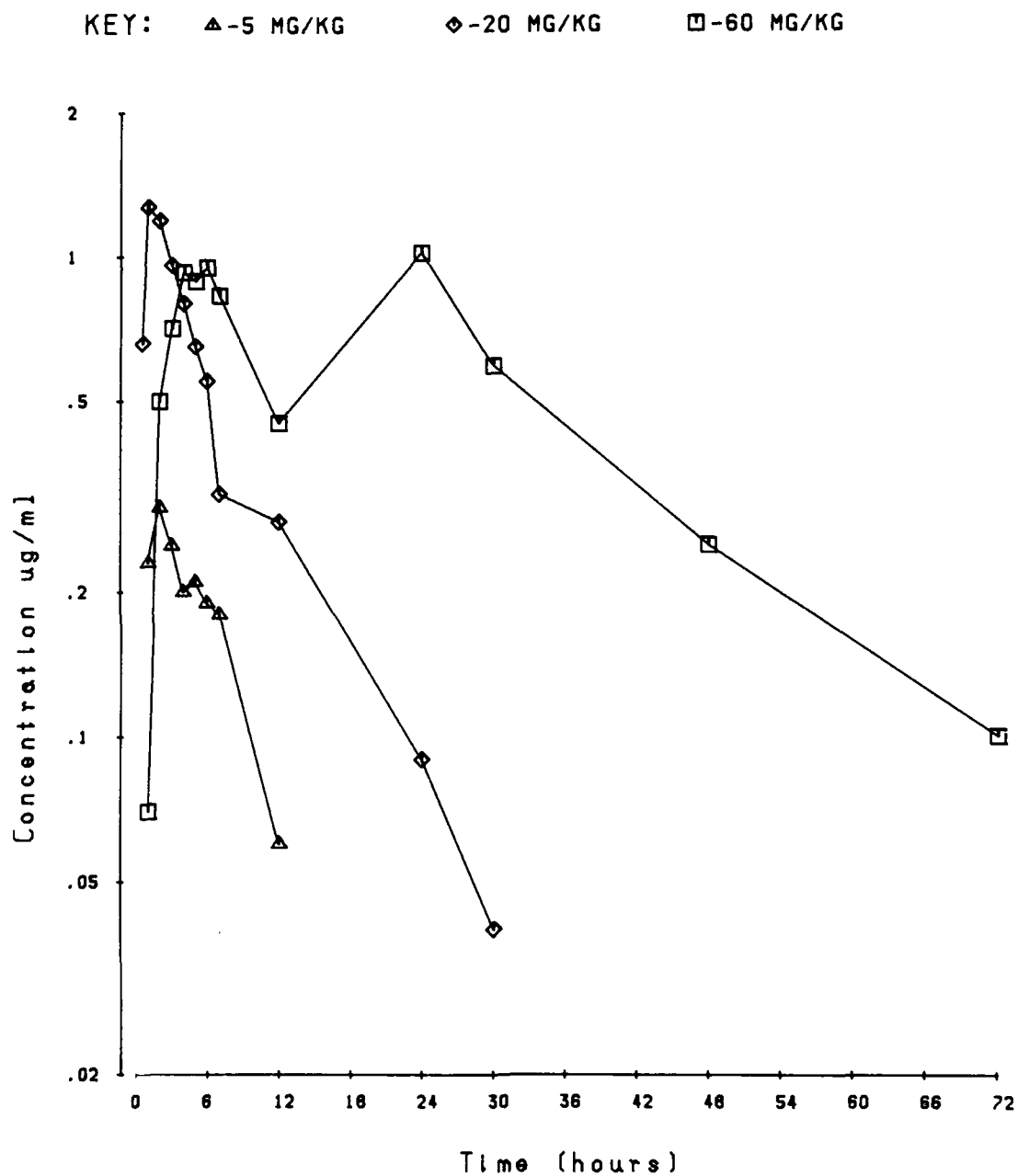


FIGURE 17

Concentrations of WR 171,669 free base in plasma after  
single oral doses of  $^{14}\text{C}$ -WR 171,669.HCl

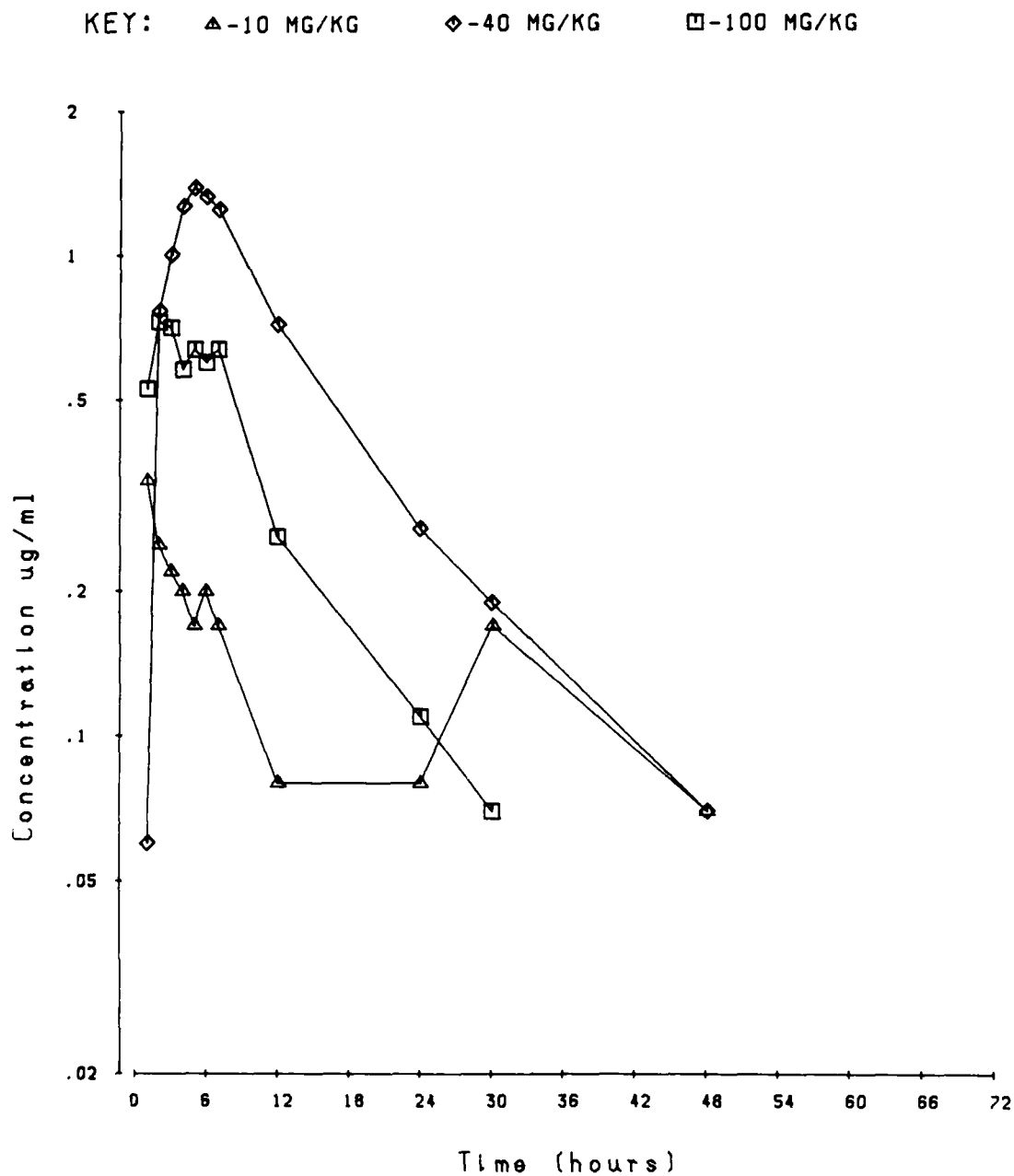


FIGURE 18

Concentrations of total radioactivity and of WR 171,669 free base  
in plasma of a dog after a single oral dose of  
 $^{14}\text{C}$ -WR 171,669.HCl at a level of 5 mg/kg

KEY:  $\diamond$ -14C-171669  $\triangle$ -TOTAL

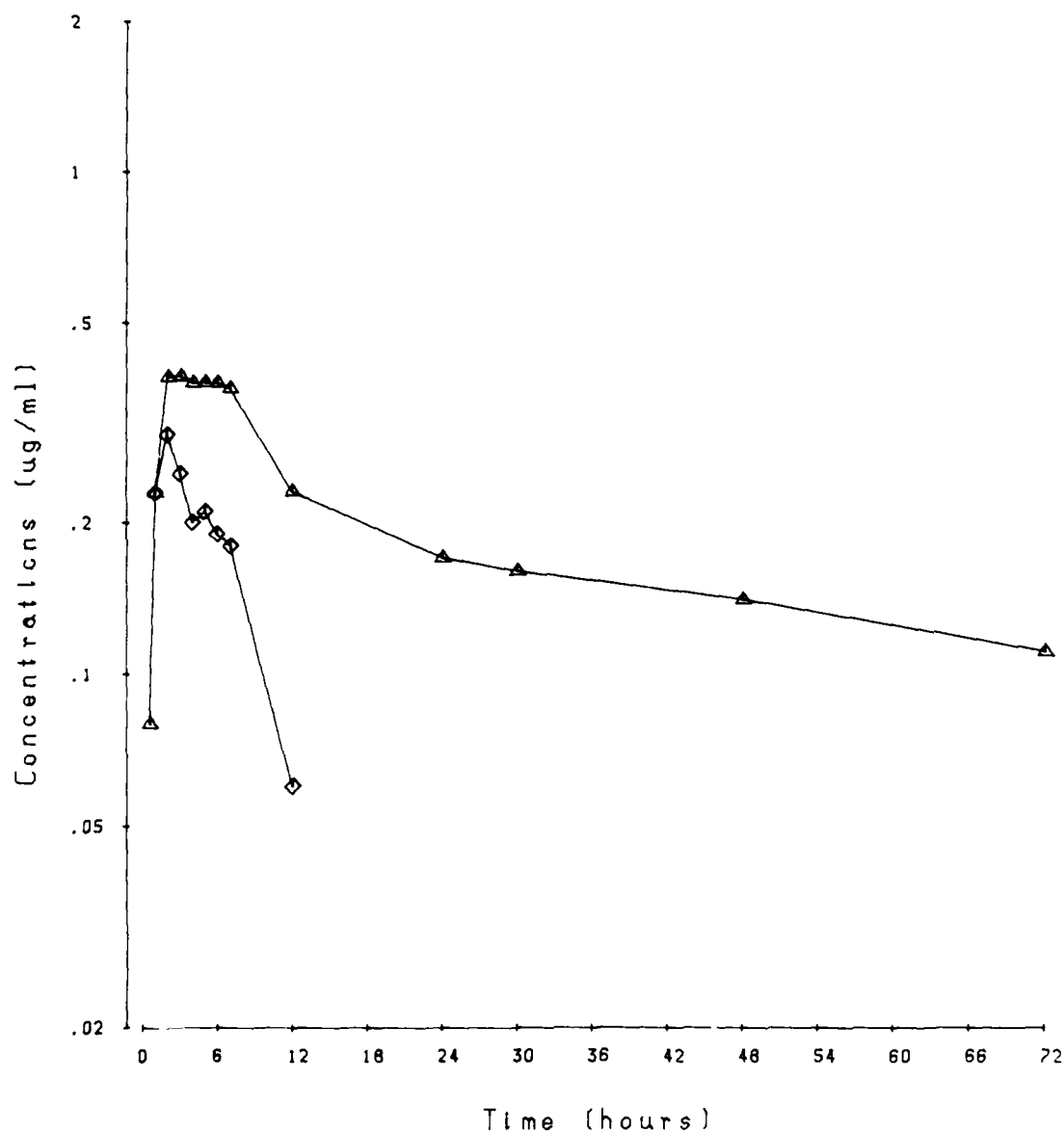




FIGURE 19

Concentrations of total radioactivity and of WR 171,669 free base  
in plasma of a dog after a single oral dose of  
 $^{14}\text{C}$ -WR 171,669.HCl at a level of 10 mg/kg

KEY:  $\diamond$ -14C-171669  $\triangle$ -TOTAL

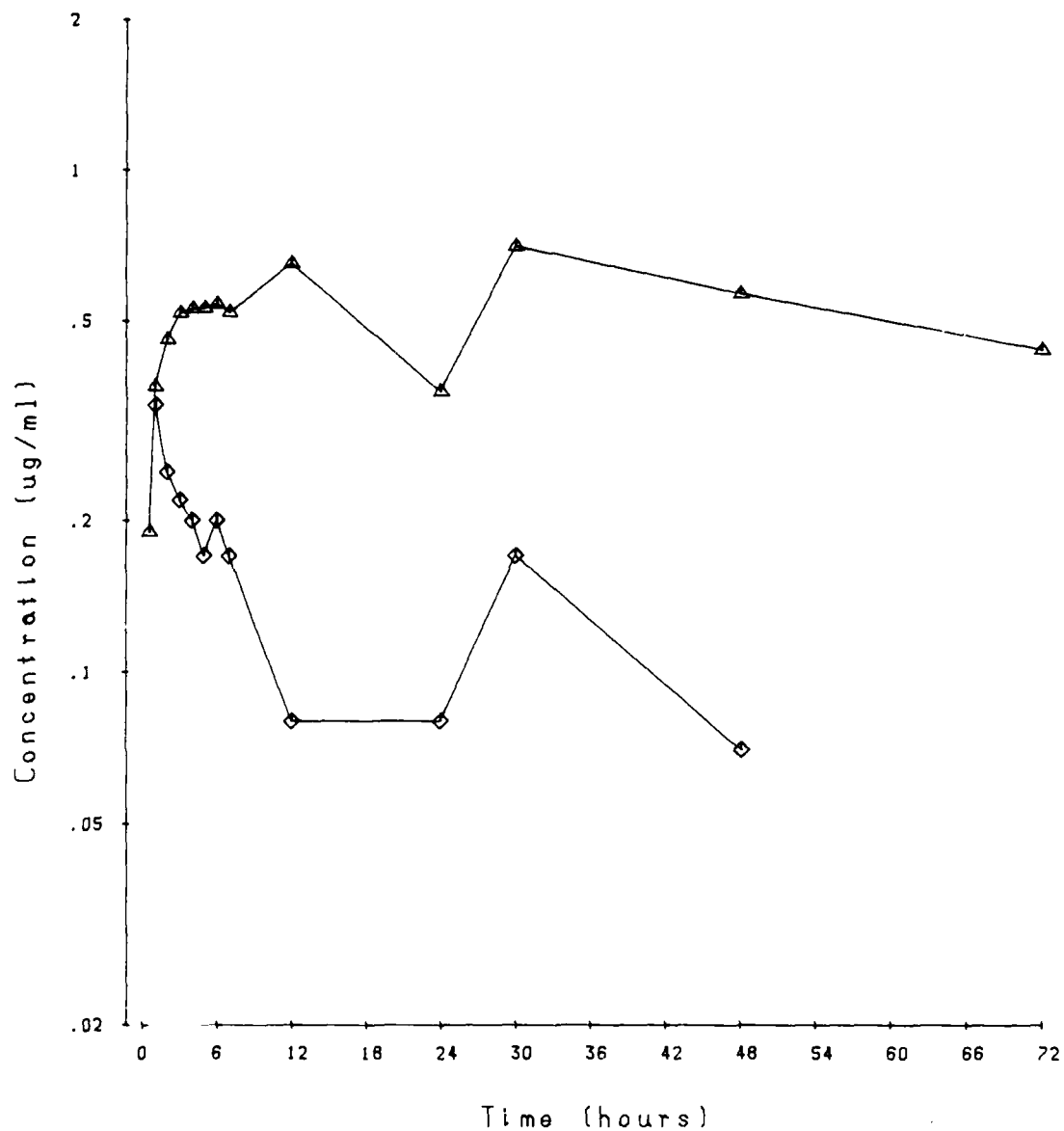


FIGURE 20

Concentrations of total radioactivity and of WR 171,669 free base  
in plasma of a dog after a single oral dose of  
 $^{14}\text{C}$ -WR 171,669.HCl at a level of 20 mg/kg

KEY:  $\diamond$ - $^{14}\text{C}$ -171669  $\triangle$ -TOTAL

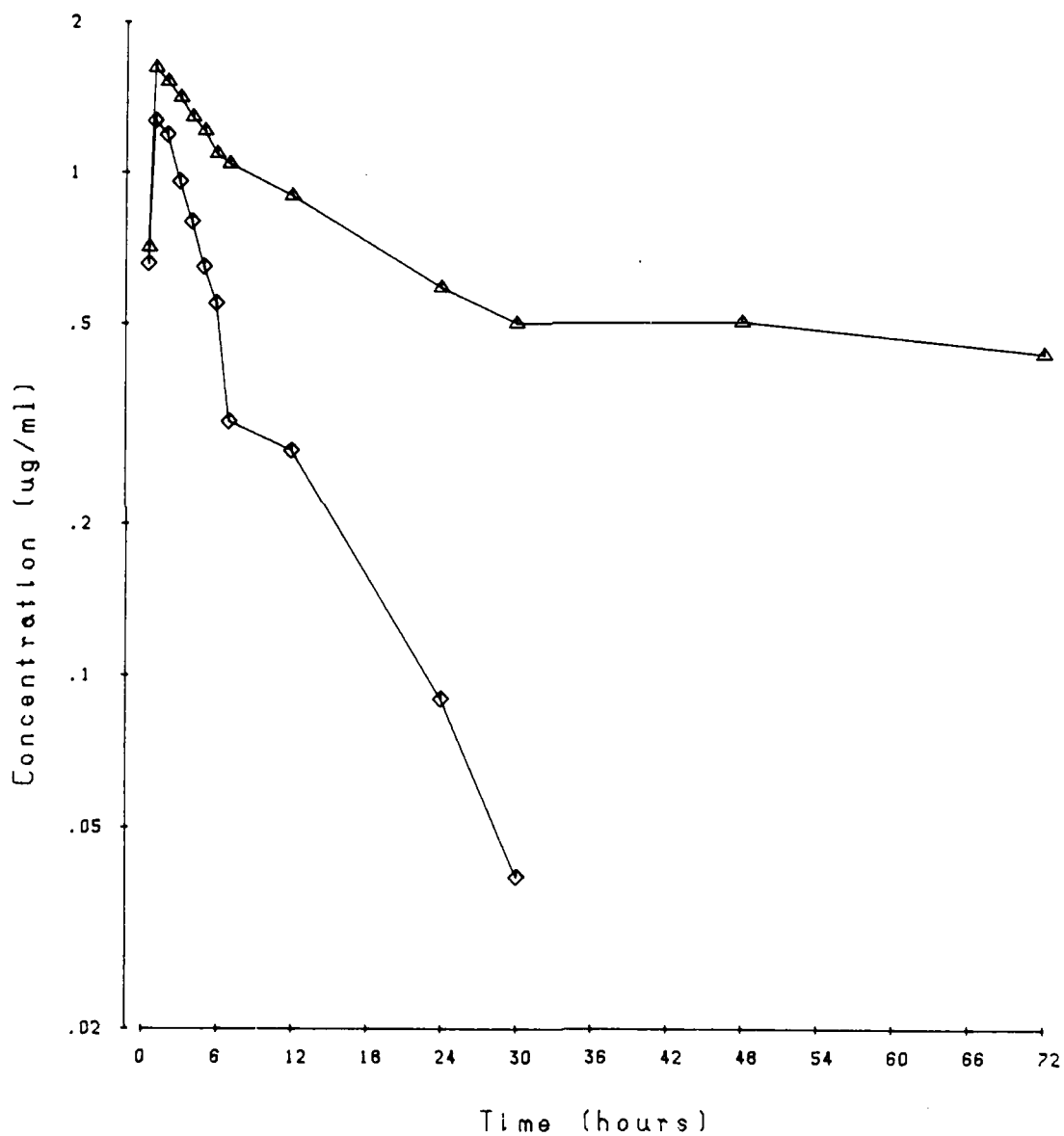


FIGURE 21

Concentrations of total radioactivity and of WR 171,669 free base  
in plasma of a dog after a single oral dose of  
 $^{14}\text{C}$ -WR 171,669.HCl at a level of 40 mg/kg

KEY:  $\diamond$ - $^{14}\text{C}$ -171669  $\triangle$ -TOTAL

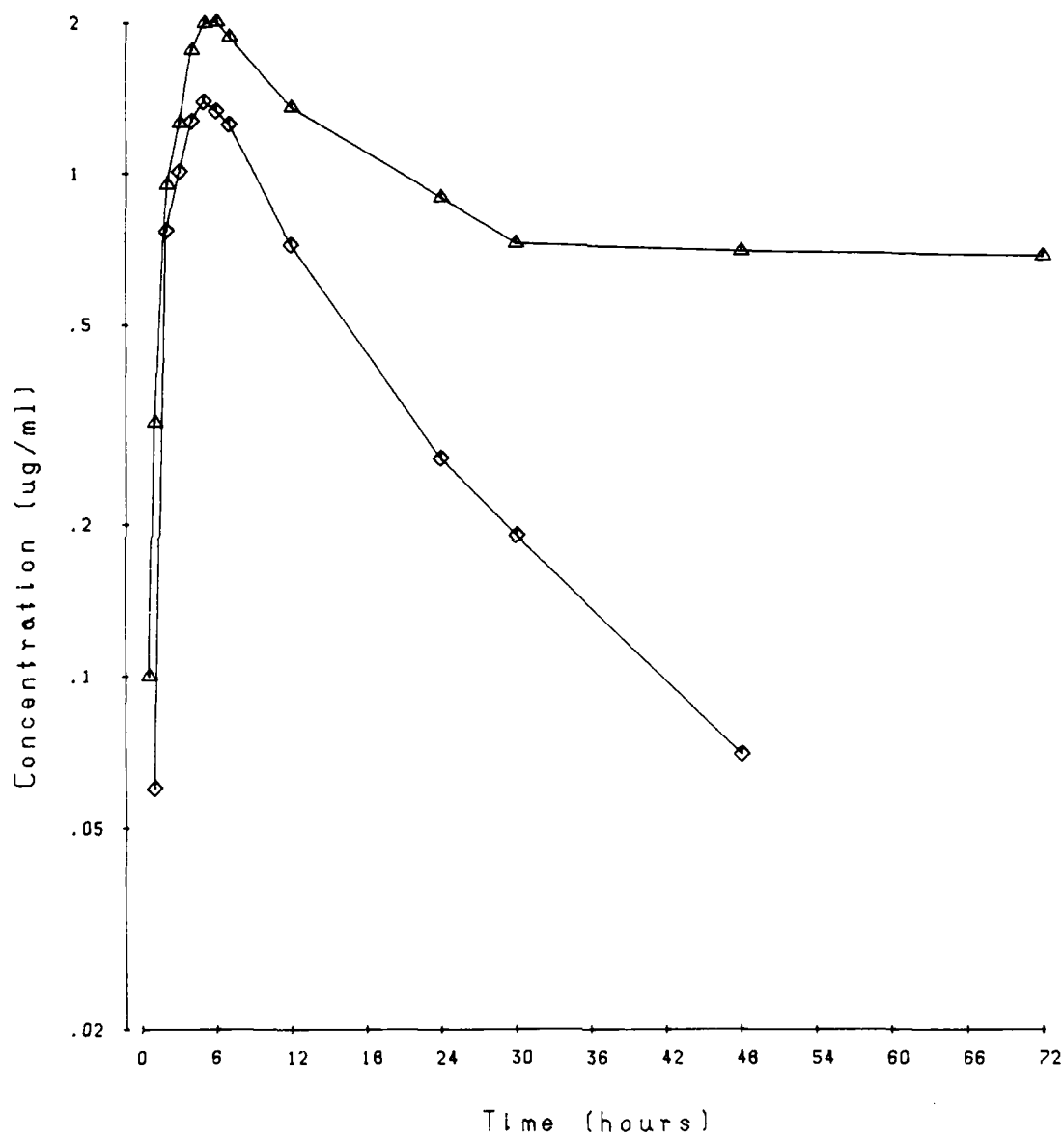


FIGURE 22

Concentrations of total radioactivity and of WR 171,669 free base  
in plasma of a dog after a single oral dose of  
 $^{14}\text{C}$ -WR 171,669.HCl at a level of 60 mg/kg

KEY:  $\diamond$ - $^{14}\text{C}$ -171669  $\triangle$ -TOTAL

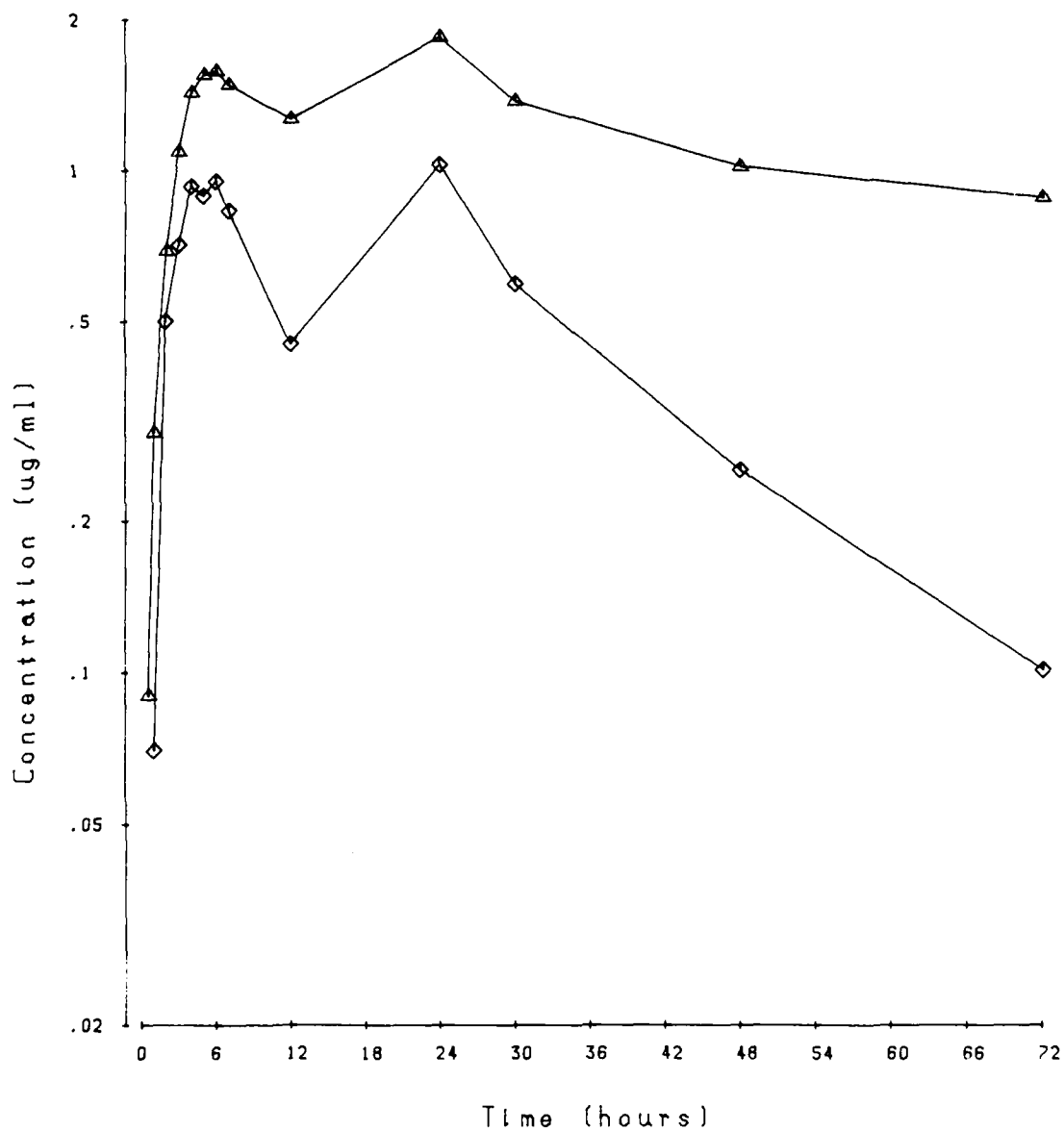


FIGURE 23

Concentrations of total radioactivity and of WR 171,669 free base  
in plasma of a dog after a single oral dose of  
 $^{14}\text{C}$ -WR 171,669.HCl at a level of 100 mg/kg

KEY:  $\diamond$ -14C-171669  $\triangle$ -TOTAL

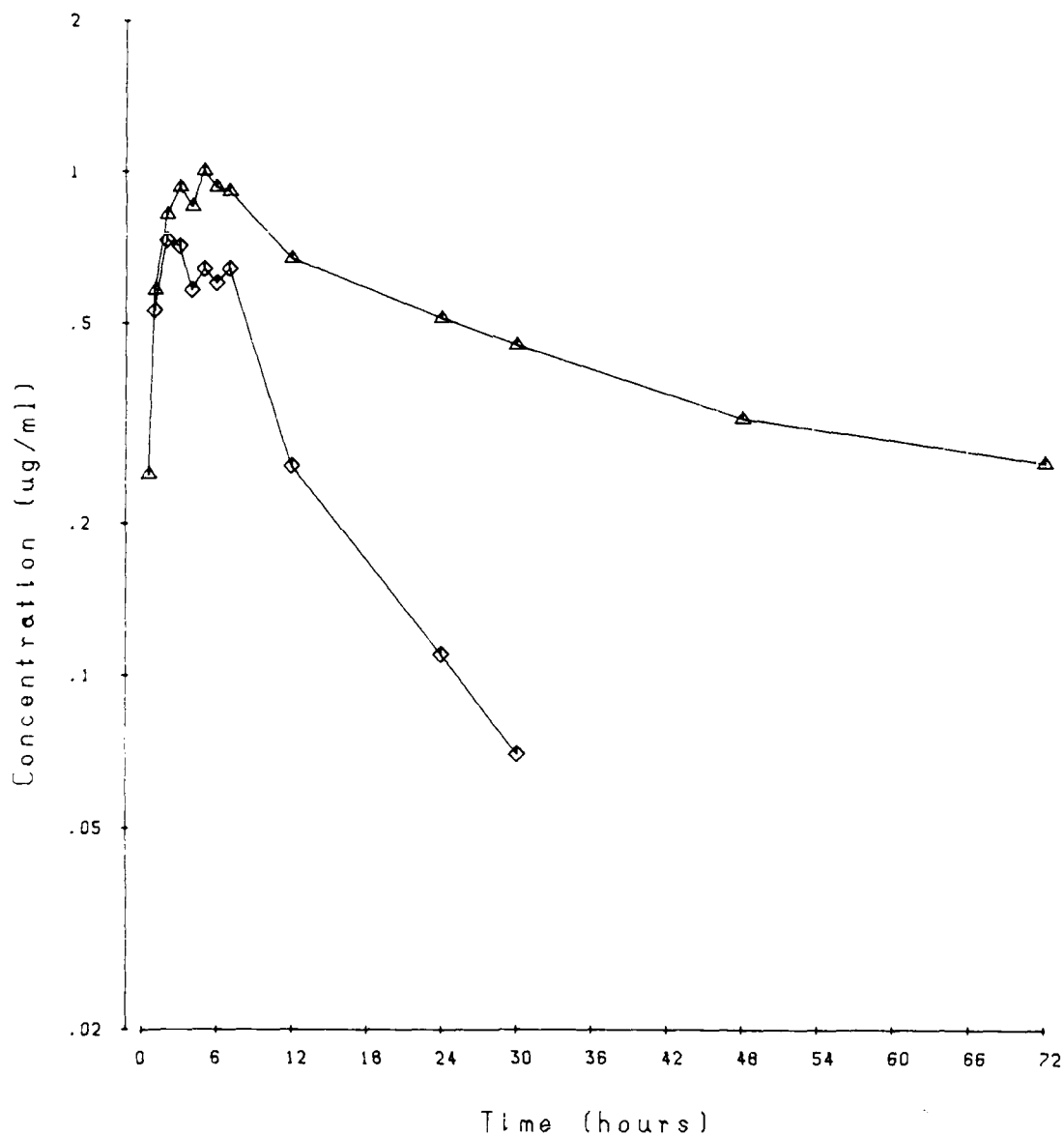


FIGURE 24

Observed points ( $\Delta$ ) and fitted curves for concentrations of total radioactivity in plasma after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 5 mg/kg

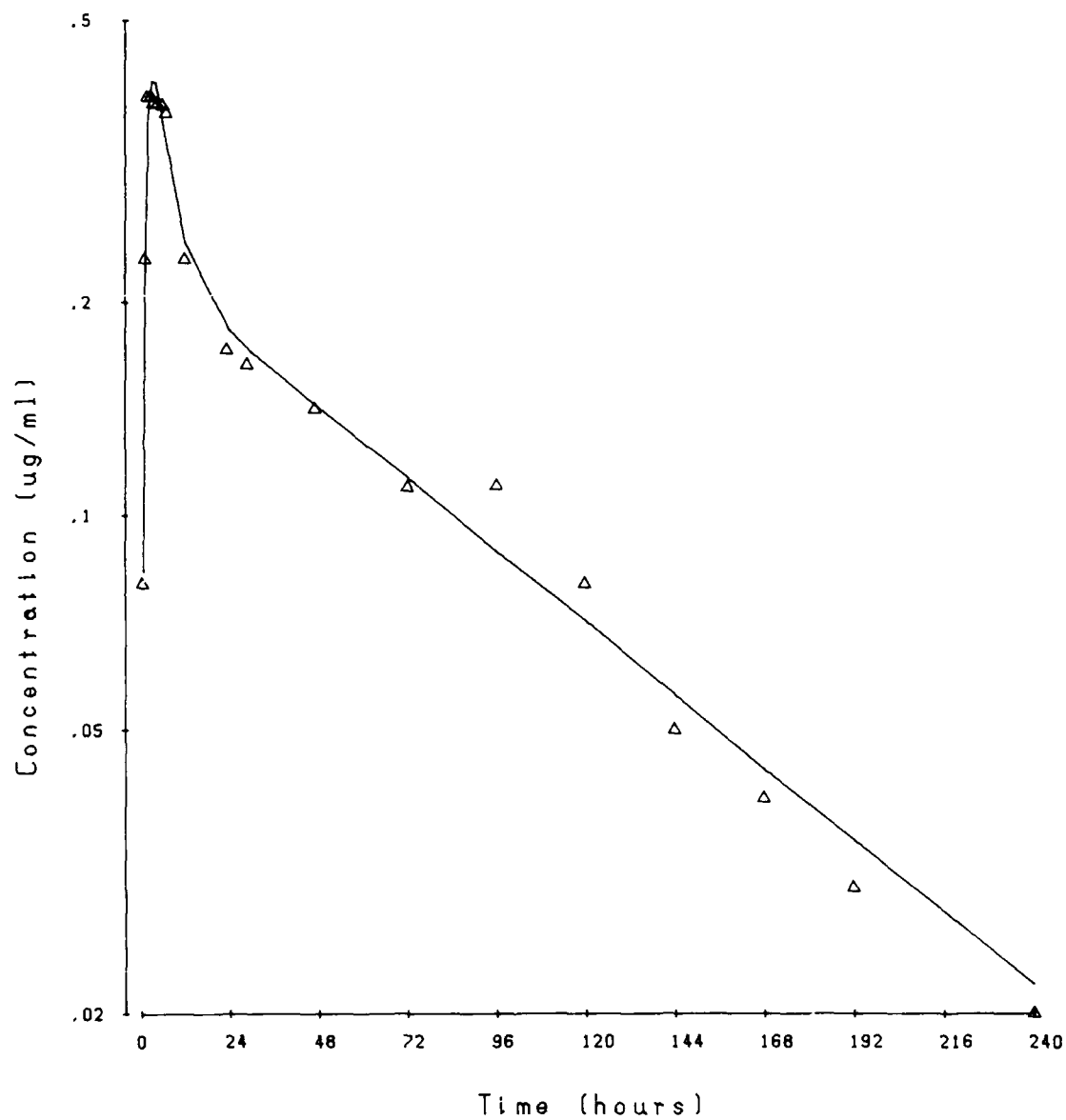


FIGURE 25

Observed points ( $\Delta$ ) and fitted curves for concentrations of total radioactivity in plasma after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 10 mg/kg

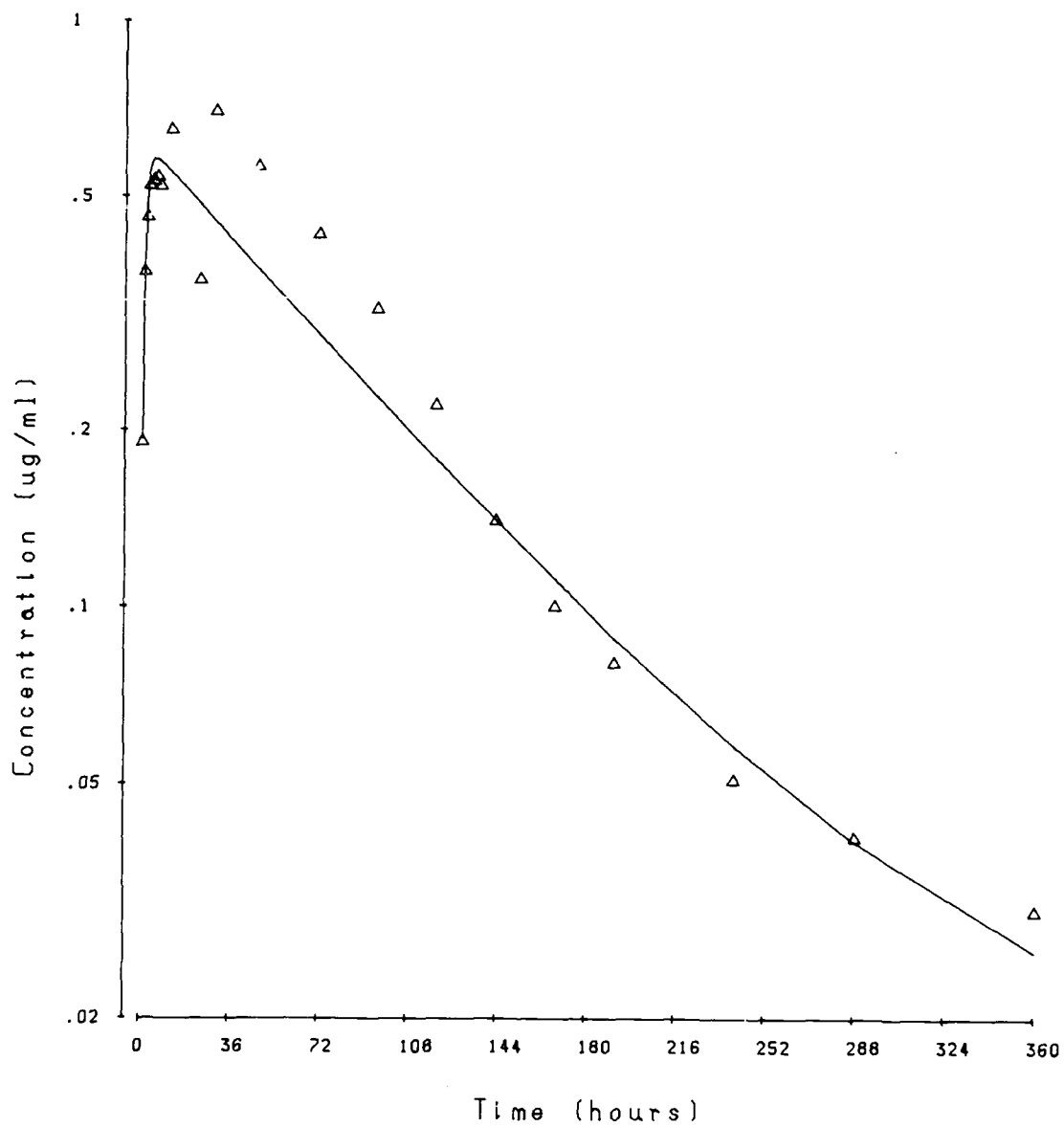


FIGURE 26

Observed points ( $\Delta$ ) and fitted curves for concentrations of total radioactivity in plasma after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 20 mg/kg

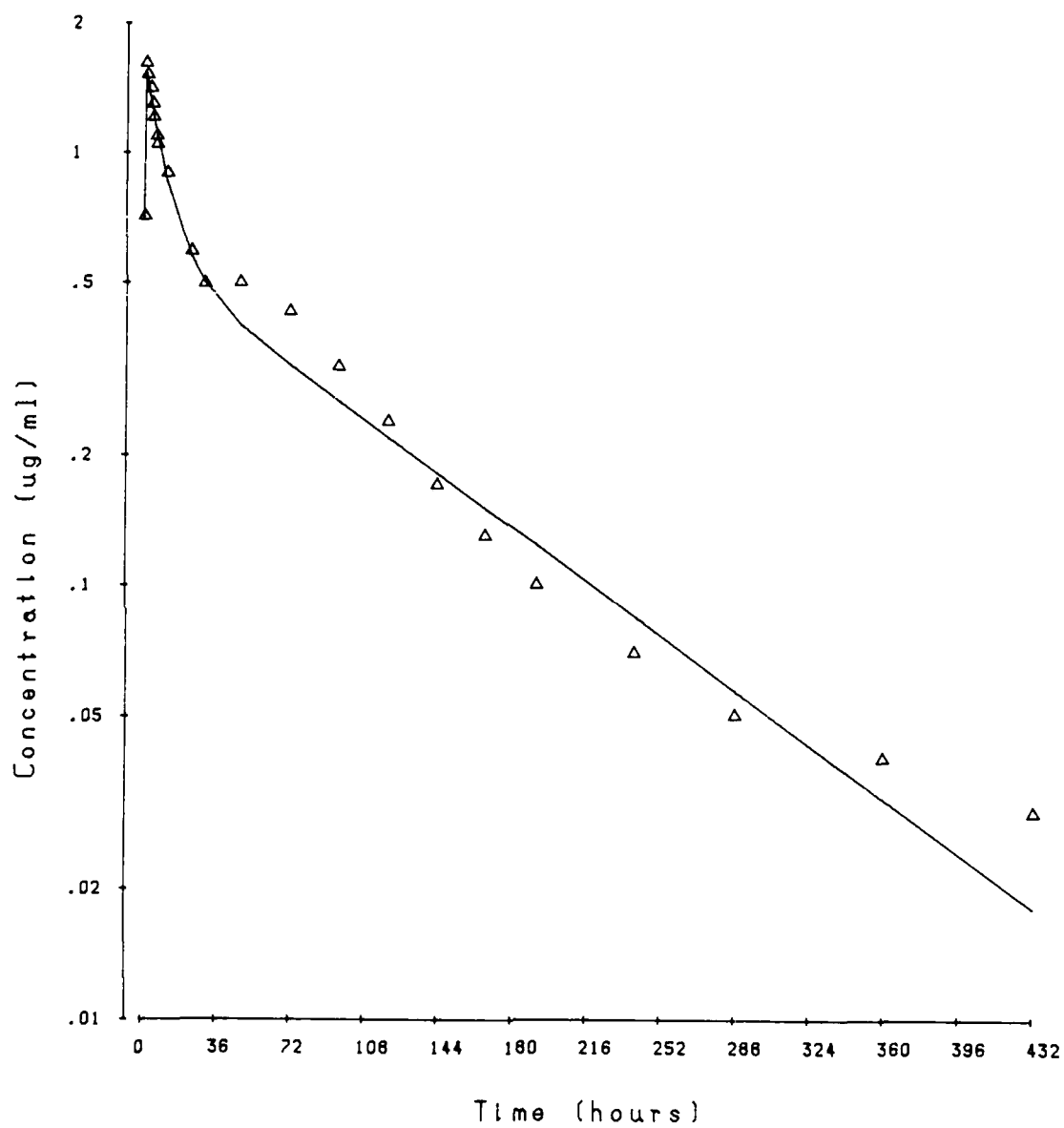




FIGURE 27

Observed points ( $\Delta$ ) and fitted curves for concentrations of total radioactivity in plasma after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 40 mg/kg

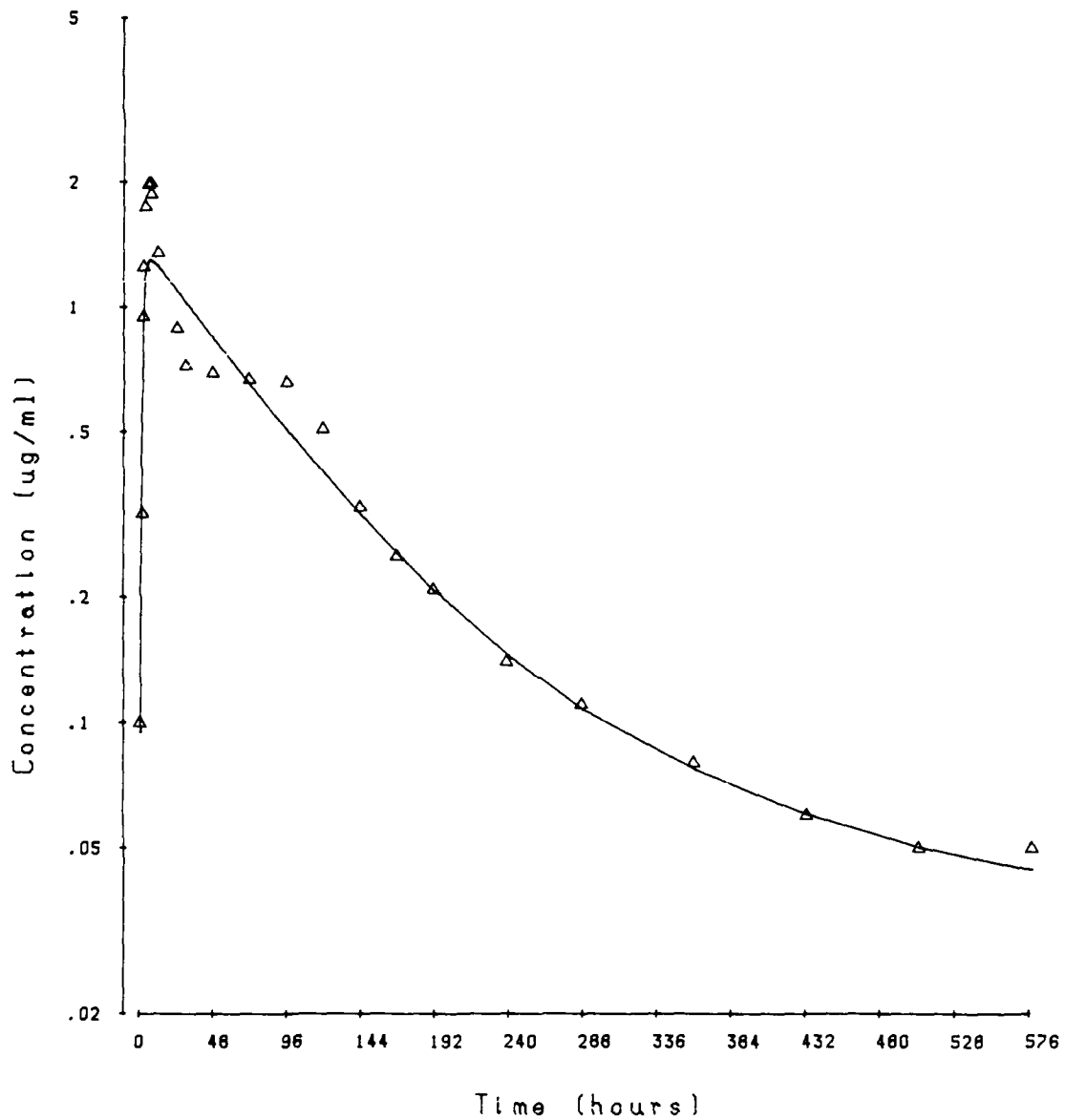


FIGURE 28

Observed points ( $\Delta$ ) and fitted curves for concentrations of total radioactivity in plasma after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 60 mg/kg

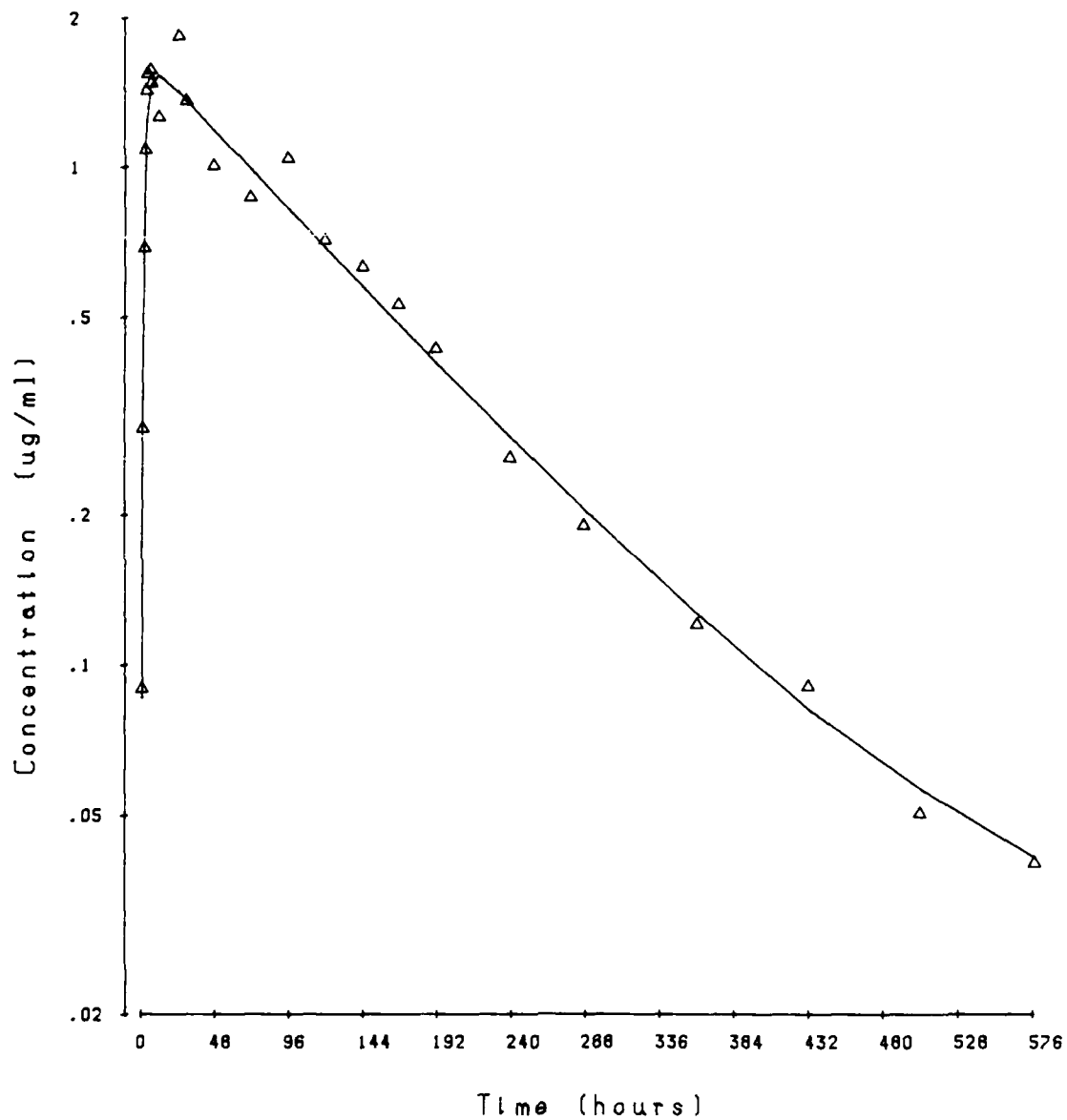


FIGURE 29

Observed points ( $\Delta$ ) and fitted curves for concentrations of total radioactivity in plasma after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 100 mg/kg

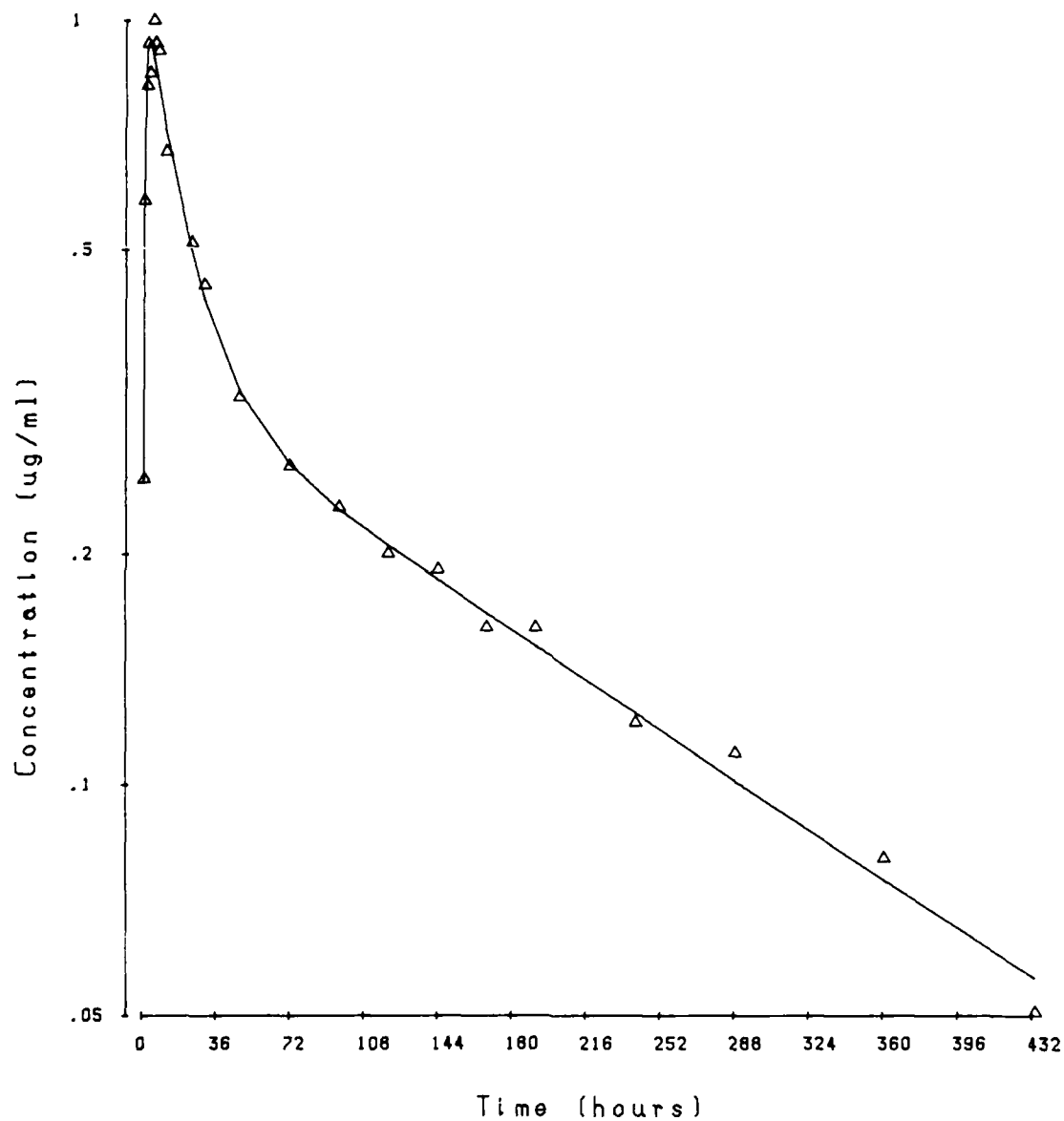


FIGURE 30

Observed points ( $\blacktriangle$ ) and fitted curves for concentrations of  
WR 171,669 free base in plasma after a single oral dose  
of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 5 mg/kg

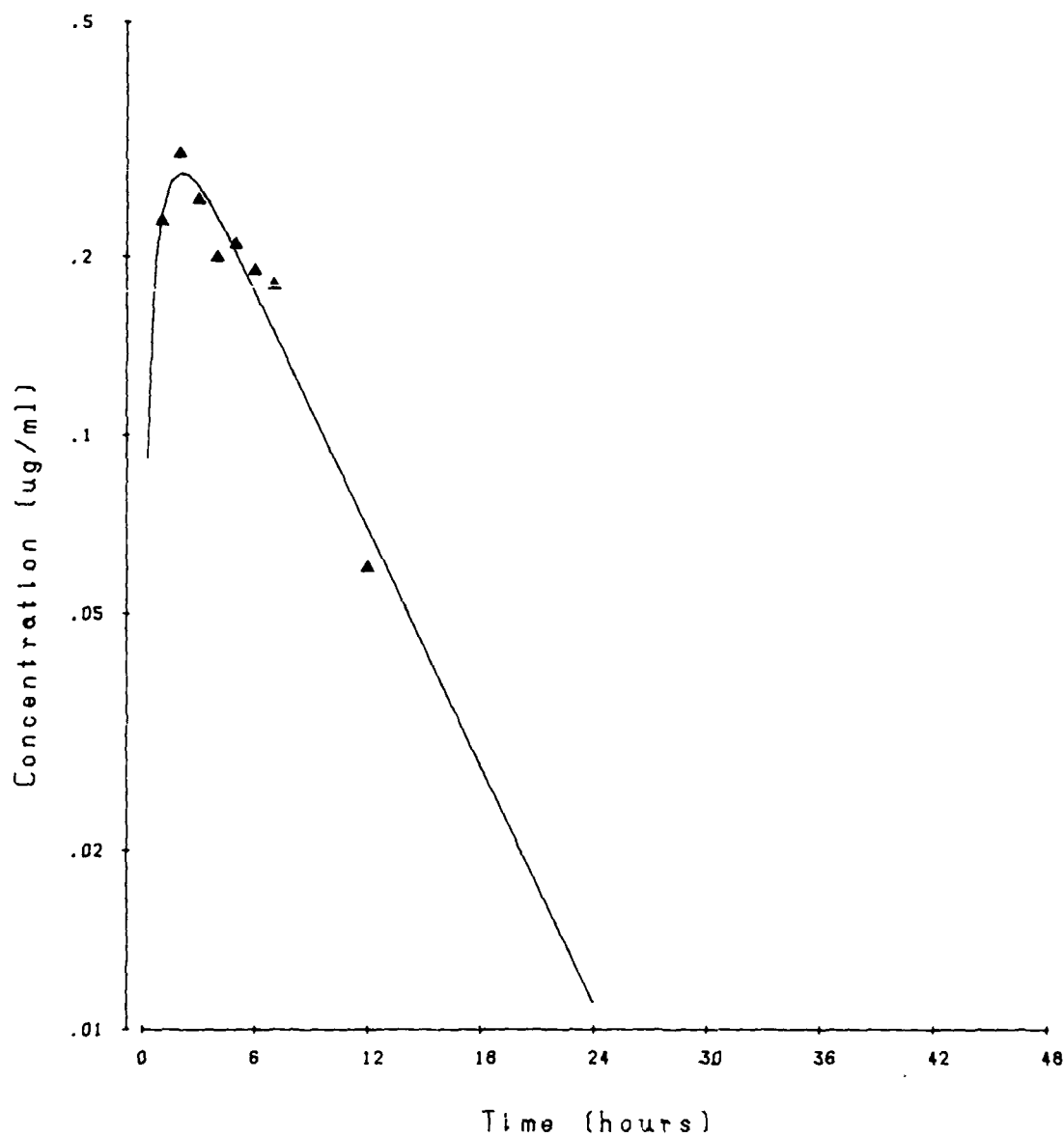


FIGURE 31

Observed points (▲) and fitted curves for concentrations of  
WR 171,669 free base in plasma after a single oral dose  
of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 10 mg/kg

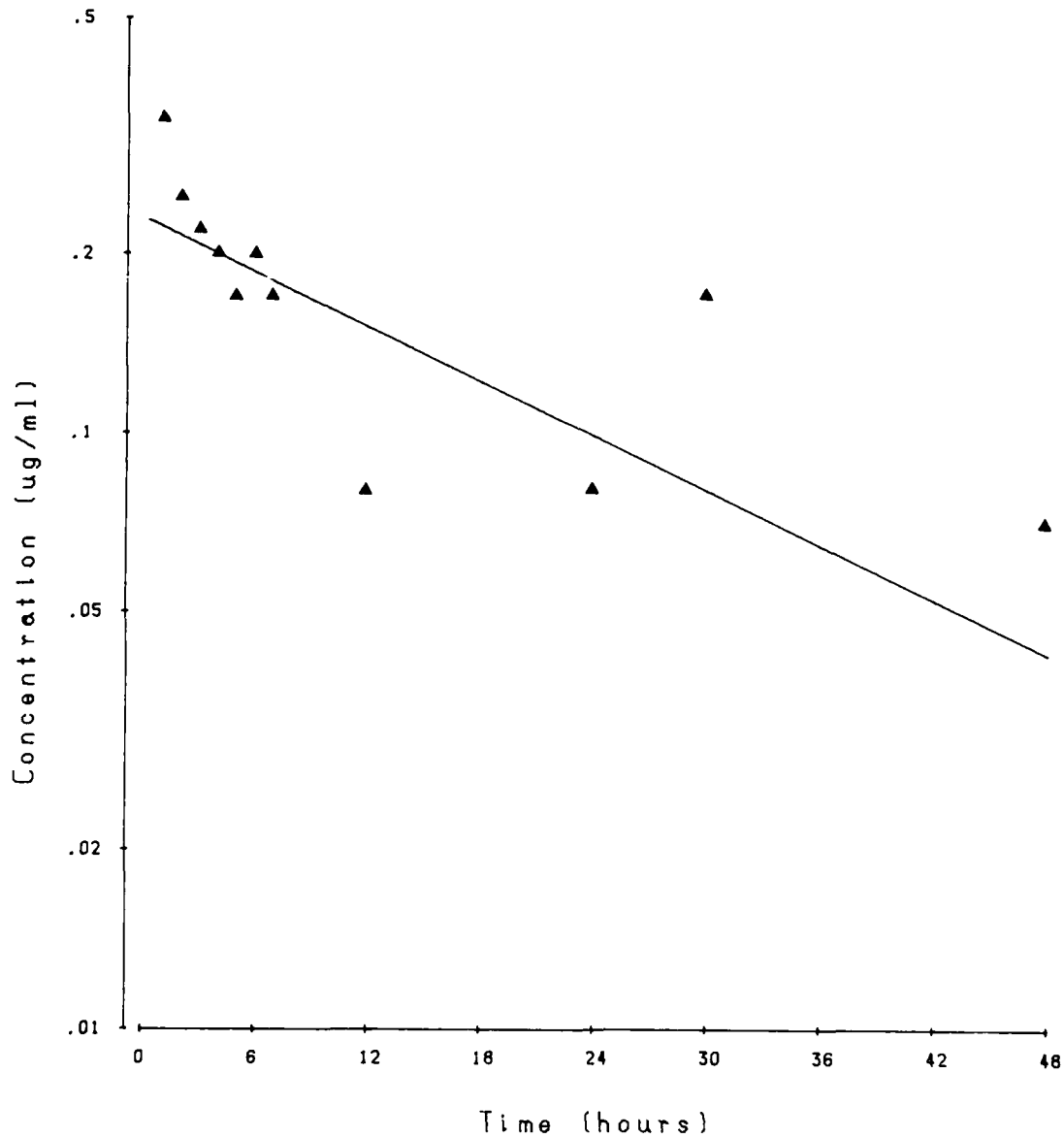


FIGURE 32

Observed points ( $\blacktriangle$ ) and fitted curves for concentrations of  
WR 171,669 free base in plasma after a single oral dose  
of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 20 mg/kg

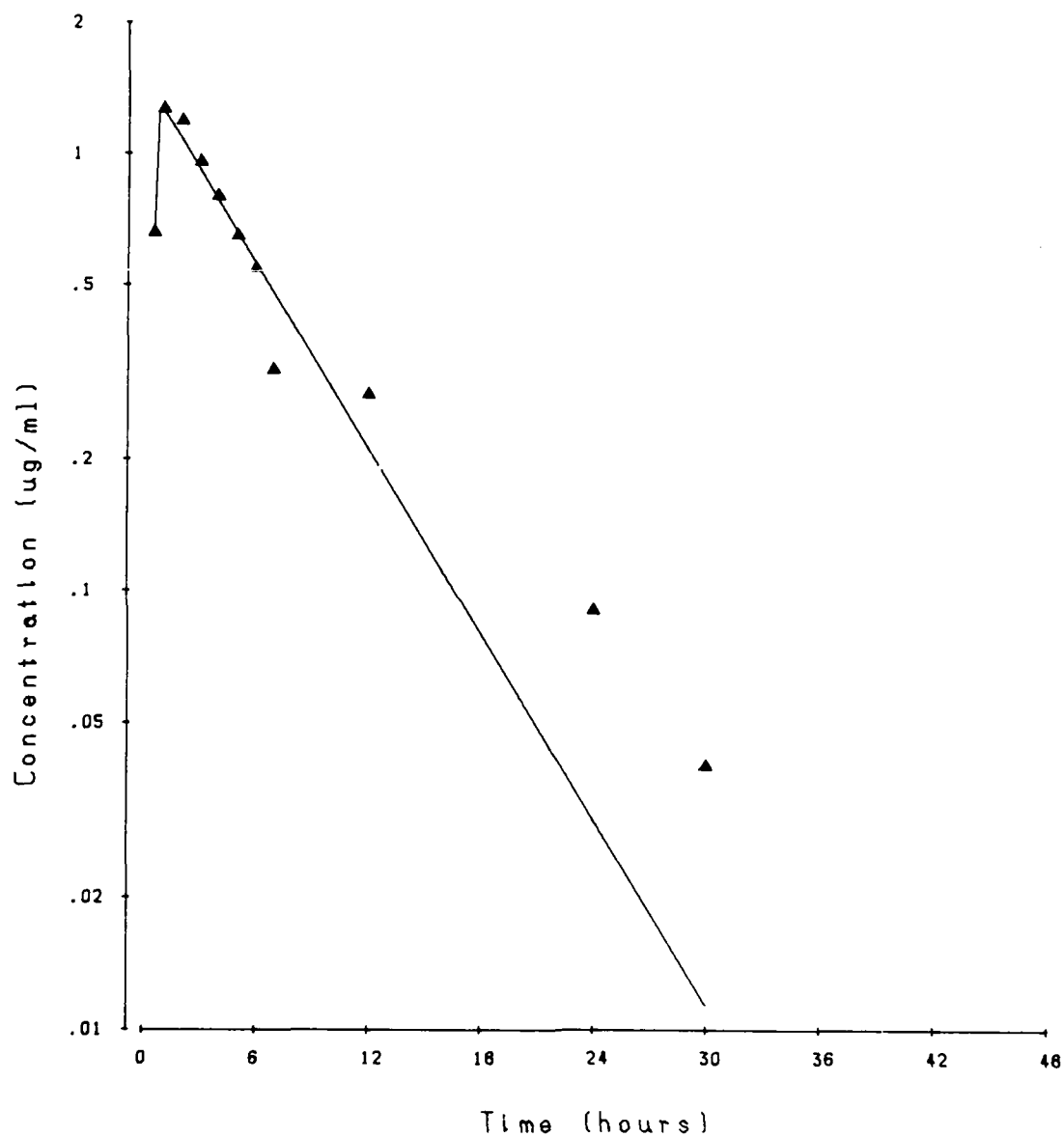


FIGURE 33

Observed points ( $\blacktriangle$ ) and fitted curves for concentrations of  
WR 171,669 free base in plasma after a single oral dose  
of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 40 mg/kg

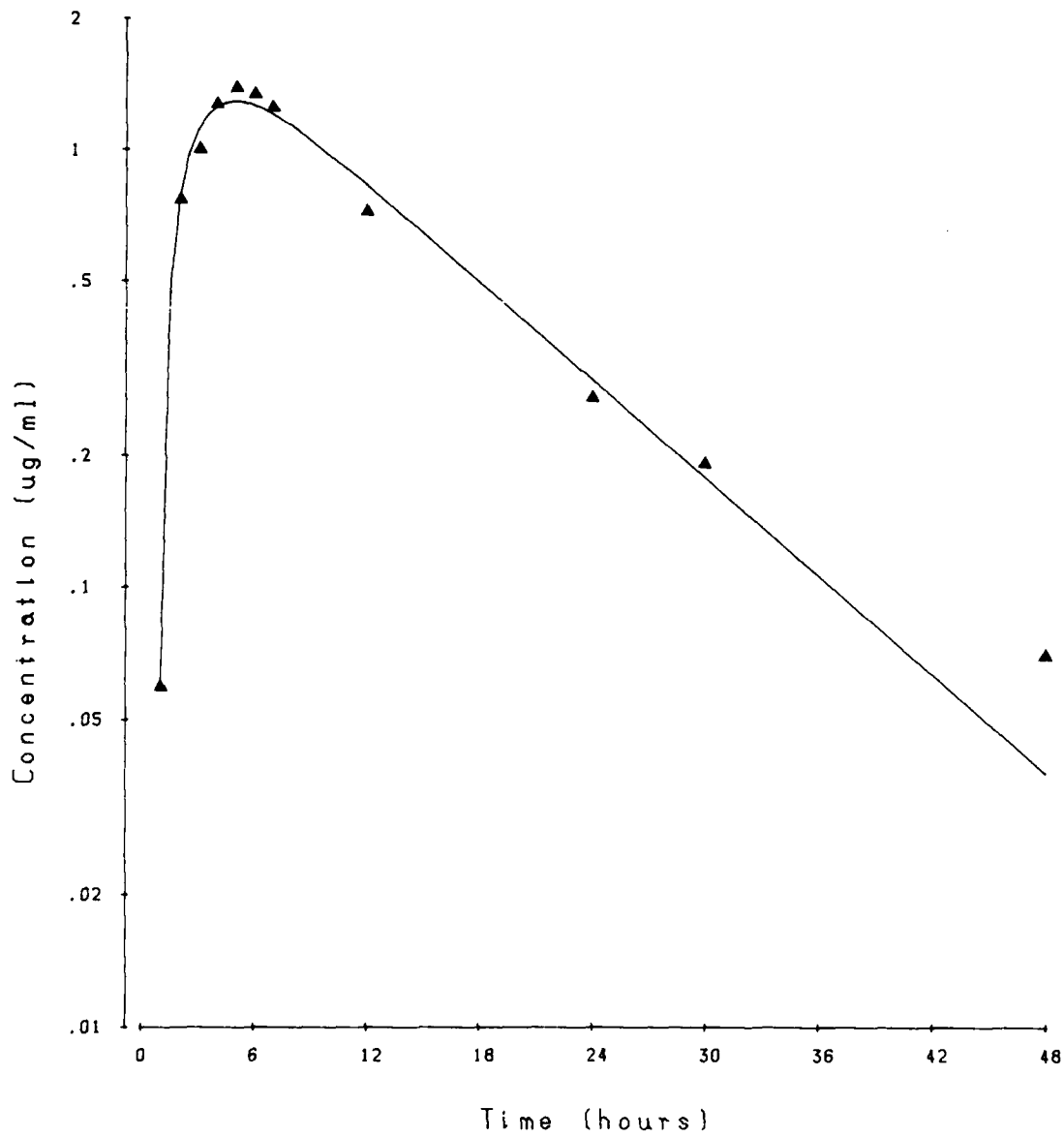


FIGURE 34

Observed points ( $\blacktriangle$ ) and fitted curves for concentrations of WR 171,669 free base in plasma after a single oral dose of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 60 mg/kg

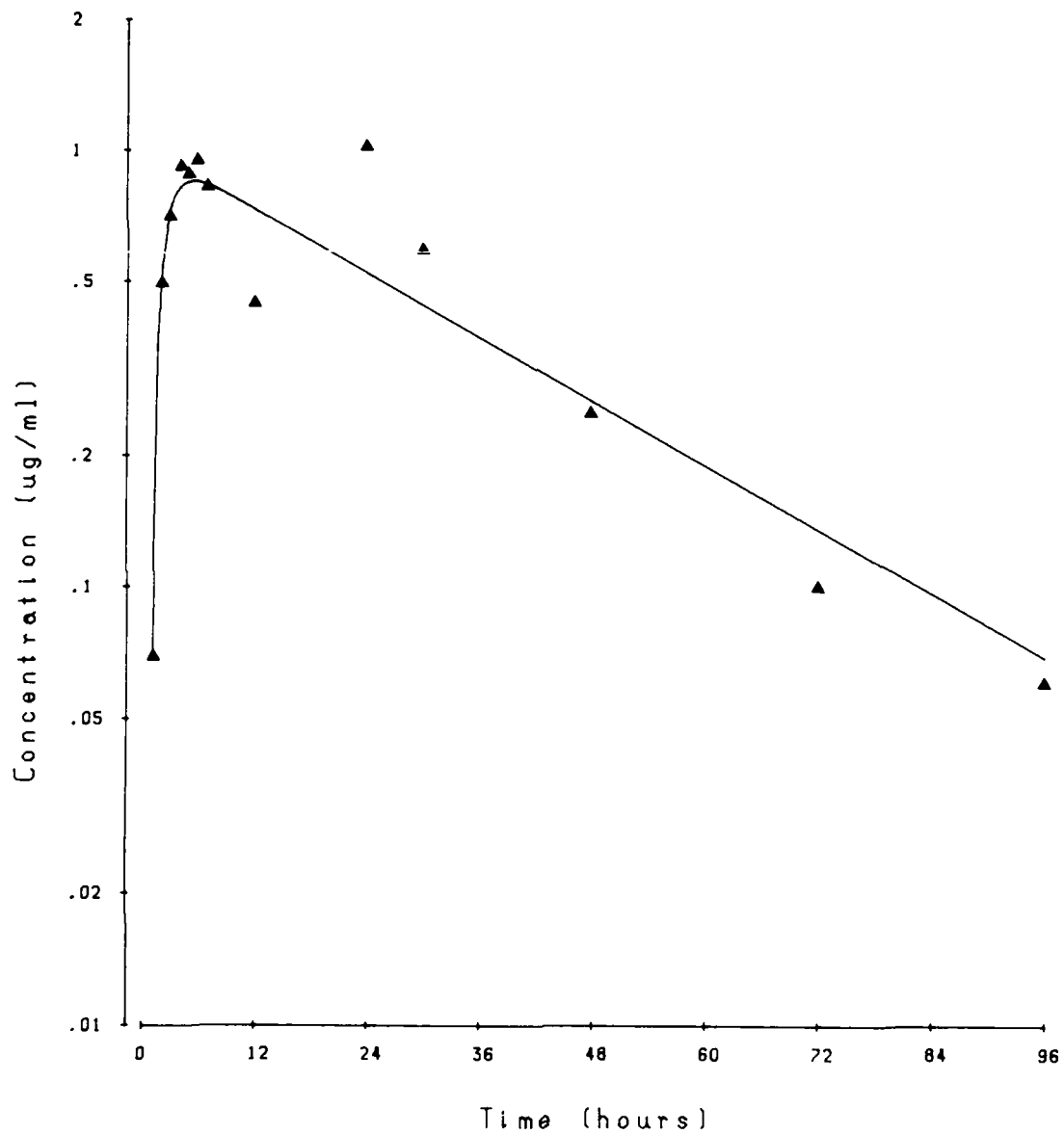




FIGURE 35

Observed points ( $\blacktriangle$ ) and fitted curves for concentrations of  
WR 171,669 free base in plasma after a single oral dose  
of  $^{14}\text{C}$ -WR 171,669.HCl at a level of 100 mg/kg

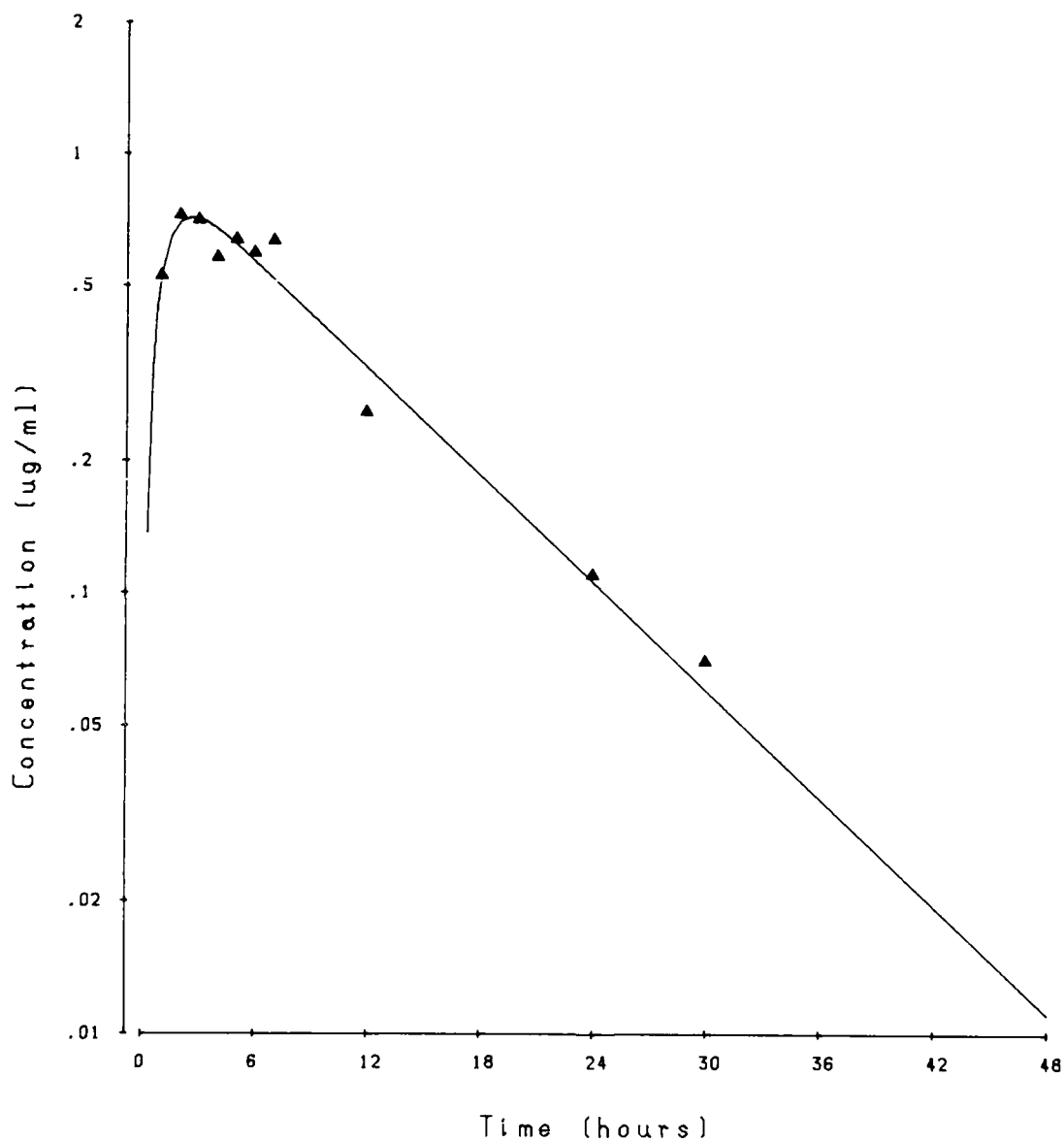


FIGURE 36

Thin-layer chromatogram autoradiograph of extracts of faeces  
Solvent system: chloroform : methanol : 35% ammonia (70:30:1, v/v)

## KEY

- A Dog 1, 0-24 hours, extract 1, after administration of  
 $^{14}\text{C}$ -WR 178,460.HCl at 20 mg/kg
- B Dog 1, 24-48 hours, extracts 1,2,3, after administration of  
 $^{14}\text{C}$ -WR 178,460.HCl at 20 mg/kg
- C Dog 1, 0-24 hours, extract 1, after administration of  
 $^{14}\text{C}$ -WR 171,669.HCl at 20 mg/kg
- D Dog 1, 24-48 hours, extracts 1,2,3, after administration of  
 $^{14}\text{C}$ -WR 171,669.HCl at 20 mg/kg
- E Dog 3, 0-24 hours, extract 1, after administration of  
 $^{14}\text{C}$ -WR 171,669.HCl at 60 mg/kg
- F Dog 3, 24-48 hours, extracts 1,2,3, after administration of  
 $^{14}\text{C}$ -WR 171,669.HCl at 60 mg/kg
- 1 WR 178,460.HCl
- 2 WR 171,669.HCl

METABOLISM AND PHARMACOKINETICS OF  
 $^{14}\text{C}$ -WR 178,460.HCl IN THE DOG

## SUMMARY

1. The purpose of this study was to carry out a pilot investigation of the metabolism and pharmacokinetics in the beagle dog, of the compound  $^{14}\text{C}$ -WR 178,460.HCl, a potential pharmacologically active metabolite of the anti-malarial drug WR 171,669.HCl.  $^{14}\text{C}$ -WR 178,460.HCl was administered orally, as a suspension in 1% aqueous carboxymethyl-cellulose solution, to a single dog at a dose level of 21 mg/kg.
2. Excretion of radioactivity in faeces accounted for 72.4% dose during the first 24 hours after dosing and for 97.2% dose in seven days. A total of only 0.1% dose was excreted in urine.
3. Plasma concentrations of radioactivity reached a plateau at 2 hours and remained within the range 0.58 to 0.73  $\mu\text{g}$  equivalents WR 178,460 free base/ml ( $\mu\text{g}/\text{ml}$ ) until 2 days. Concentrations then appeared to decline biphasically with an initial more rapid phase ( $t_{1/2}$  52.6 h  $\pm$  9.5 h S.E.) followed by a slower terminal phase for which a half-life could not be precisely determined. By 21 days the plasma concentration had declined to 0.02  $\mu\text{g}$  equiv./ml.
4. Whole-blood concentrations of radioactivity were slightly higher than the corresponding plasma concentrations during the period from 5 to 24 hours after dosing (maximum blood : plasma ratio 1.15) but then declined more rapidly to be below 0.06  $\mu\text{g}/\text{ml}$  at 8 days.

TABLE 1

Excretion of radioactivity after oral administration  
of  $^{14}\text{C}$ -WR 178,460.HCl to a beagle dog at a dose  
level of 21 mg/kg

Results are expressed as % dose

Time (hours)	Radioactivity excreted			Cumulative excretion			
	Urine	Faeces	Cage wash	Urine	Faeces	Cage wash	Total
0- 24	0.05	72.4	0.20	0.05	72.4	0.20	72.6
24- 48	0.02	8.4	0.12	0.07	80.8	0.32	81.2
48- 72	0.02	7.2	0.12	0.09	88.0	0.44	88.5
72- 96	0.009	4.9	0.09	0.10	92.9	0.53	93.6
96-120	0.007	2.1	0.11	0.11	95.0	0.64	95.8
120-144	0.006	1.2	0.03	0.12	96.2	0.67	97.0
144-168	0.004	1.0	0.01	0.12	97.2	0.68	98.0

TABLE 2

Concentrations of radioactivity in plasma and whole-blood  
after oral administration of  $^{14}\text{C}$ -WR 178,460.HCl to a  
beagle dog at a dose level of 21 mg/kg

Time	$\mu\text{g}$ equivalents WR 178,460*/ml		% dose/litre	
	Plasma	Whole-blood†	Plasma	Whole-blood†
15 min	0.07	0.07	0.03	0.03
30 min	0.20	0.18	0.08	0.08
1 hr	0.30	0.29	0.12	0.12
2 hrs	0.64	0.52	0.26	0.21
3 hrs	0.66	0.62	0.27	0.25
4 hrs	0.68	0.66	0.28	0.27
5 hrs	0.66	0.69	0.27	0.28
6 hrs	0.64	0.69	0.26	0.28
7 hrs	0.61	0.70	0.25	0.29
12 hrs	0.58	0.67	0.24	0.27
24 hrs	0.71	0.72	0.29	0.29
30 hrs	0.73	0.70	0.30	0.29
2 days	0.66	0.57	0.27	0.23
3 days	0.48	0.31	0.20	0.13
4 days	0.30	0.24	0.12	0.10
5 days	0.20	0.14	0.08	0.06
6 days	0.14	0.08	0.06	0.03
7 days	0.10	0.07	0.04	0.03
8 days	0.09	< 0.06	0.04	< 0.03
10 days	0.05	< 0.06	0.02	< 0.03
12 days	0.05	< 0.06	0.02	< 0.03
14 days	0.04	< 0.06	0.02	< 0.03
18 days	0.03	< 0.06	0.01	< 0.03
21 days	0.02	< 0.06	0.009	< 0.03

\* Calculated as free base

† A value of 1.052, for the specific gravity of  
dog blood, was used in calculation of these  
results from data in Appendix 6

TABLE 3

Extraction/ of radioactivity from faeces samples up to 72 hours  
after oral administration of  $^{14}\text{C}$ -WR 178,460.HCl to a  
beagle dog at a dose level of 21 mg/kg

Results are expressed as % faecal radioactivity

Time period (hours)	Extract 1	Extract 2	Extract 3	Total*
0 - 24	24	31	21	75 (54.6)
24 - 48	19	23	14	57 (4.8)
48 - 72	9	30	17	55 (4.0)

\* Total is calculated from individual results to one  
decimal place and then rounded to nearest whole number  
Figures in brackets are % dose

/ Extract 1; methanol, Extract 2, 3; methanol : diethylamine  
9 : 1, v/v

TABLE 4

Radioactivity in one minute\* fractions of hplc eluate after injection of extracts of faeces collected up to 72 hours after oral administration of  $^{14}\text{C}$ -WR 178,460.HCl to a beagle dog at a dose level of 21 mg/kg

Time interval (hours)	Fraction numbers (inclusive)	% eluted radioactivity		Total % dose (all 3 extracts)
		Extract 1	Extract 2 + 3	
0 - 24	3 - 6	14.4	7.7	5.4
	9 - 10	3.9	1.6	1.3
	12 - 13*	81.6	89.1+	47.0
	Others	-	1.6	0.9
24 - 48	3 - 6	74.6	76.9	3.6
	9 - 10	12.0	6.7	0.4
	12 - 13*	13.4	9.8 $\frac{1}{2}$	0.5
	Others	-	6.6	0.2
48 - 72	3 - 6	83.1	87.2	3.4
	9 - 10	16.9	5.5	0.3
	12 - 13*	<5.0	4.2 $\frac{1}{2}$	0.1 - 0.2
	Others	-	3.1	0.1

- \* Corresponds to WR 178,460
- + Includes 1.5% from fractions 14 and 15
- $\frac{1}{2}$  Fractions 11, 12 (not 13)
- \* For results in detail see Appendix 3



TABLE 5

Concentrations of WR 178,460\* free base in plasma  
after oral administration of  $^{14}\text{C}$ -WR 178,460.HCl  
to a beagle dog at a dose level of 21 mg/kg

Time (hours)	Concentration ( $\mu\text{g/ml}$ )	Proportion of total plasma radioactivity (%)
1	0.15	50.9
2	0.37	57.6
3	0.39	59.5
4	0.40	59.5
5	0.40	61.0
6	0.37	57.1
7	0.34	55.0
12	0.32	54.6
24	0.23	32.2
30	0.18	24.7
48	0.08	12.9
72	<0.04	<8.3

\* Refers to radioactivity eluting from an hplc column  
with the same retention time as WR 178,460

Results are not corrected for recovery

TABLE 6

Radioactivity in one minute fractions\* of hplc eluate after  
 injection of extracts of plasma collected up to 72 hours  
 after oral administration of  $^{14}\text{C}$ -WR 178,460.HCl to a  
 beagle dog at a dose level of 21 mg/kg

Results are expressed as % eluted radioactivity

Time (hours)	Fractions 3 - 5	Fractions 8 - 10	Fractions 11 - 13*
1	49.1	<9.6	50.9
2	36.2	6.2	57.6
3	35.2	5.3	59.6
4	34.3	6.2	59.5
5	34.0	5.0	61.0
6	37.8	5.1	57.1
7	39.5	5.6	55.0
12	35.9	9.5	54.6†
24	59.8	8.0	32.2
30	65.5	9.8	24.7
48	78.9	8.2	12.9
72	100	<8.3	<8.3

\* For results in detail see Appendix 4

† Fractions 13 - 14

\* Corresponds to WR 178,460

FIGURE 1

Concentrations of radioactivity in plasma and whole-blood up to 48 hours after oral administration of  $^{14}\text{C}$ -WR 178,460.HCl to a beagle dog

□-□ plasma  
■-■ whole-blood

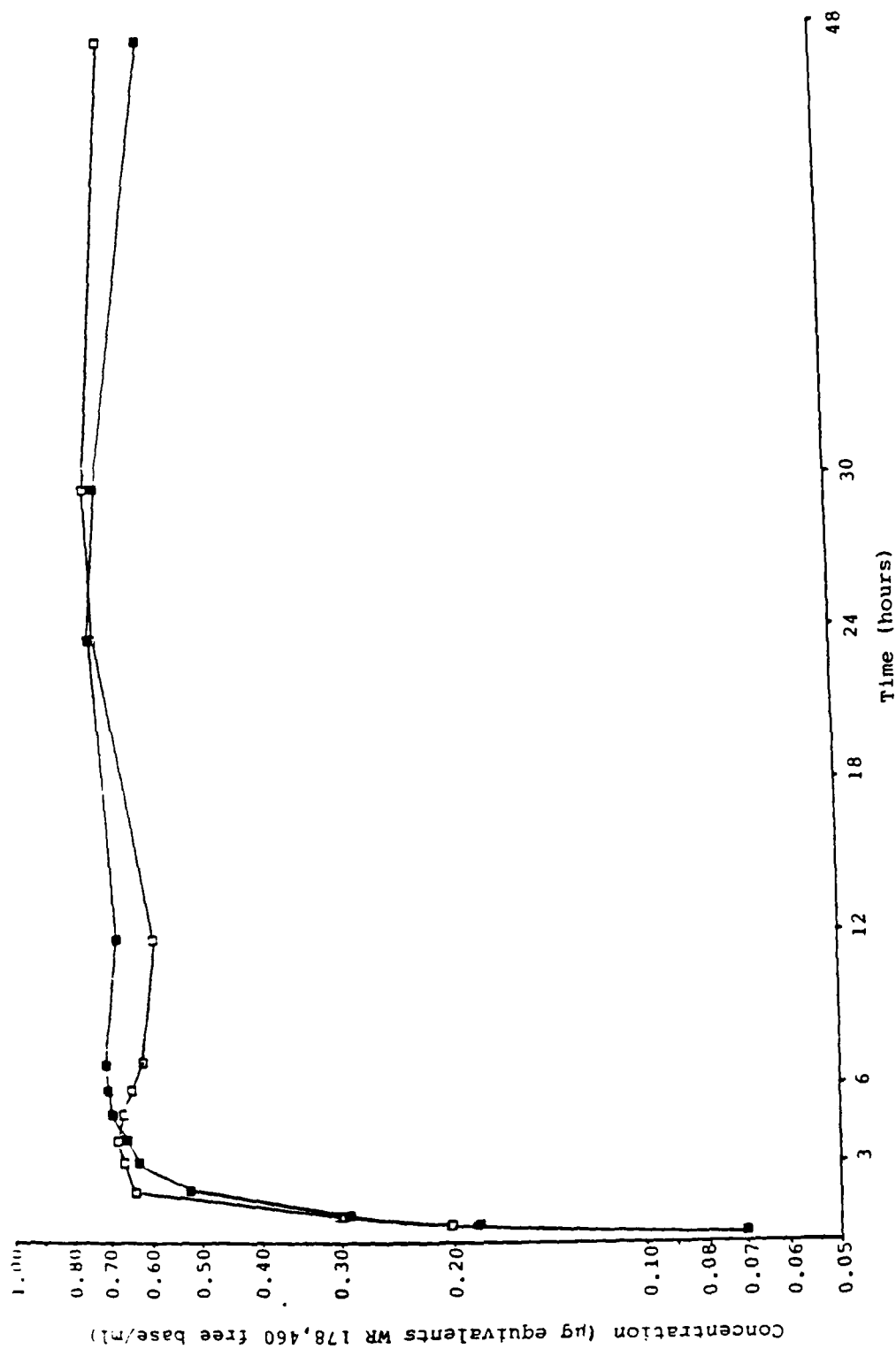


FIGURE 2

Concentrations of radioactivity in plasma and whole-blood up to 21 days  
after oral administration of  $^{14}\text{C}$ -WR 178,460.HCl to a beagle dog

□-□ plasma  
■-■ whole-blood

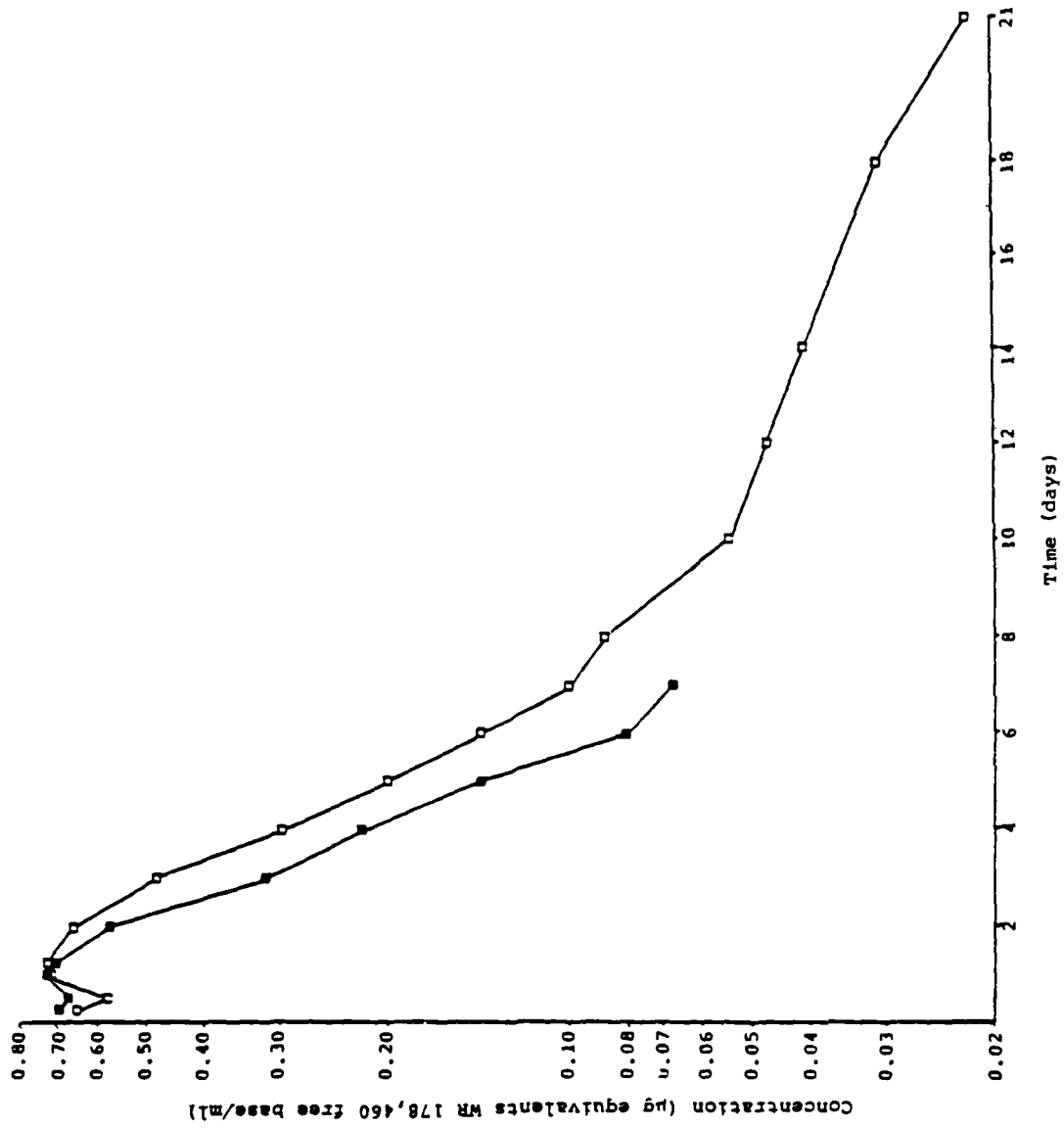


FIGURE 3

Concentrations of radioactivity and of WR 178,460 in plasma after administration of  $^{14}\text{C}$ -WR 178,460.HCl to a beagle dog

□-□ radioactivity  
▲-▲ WR 178,460

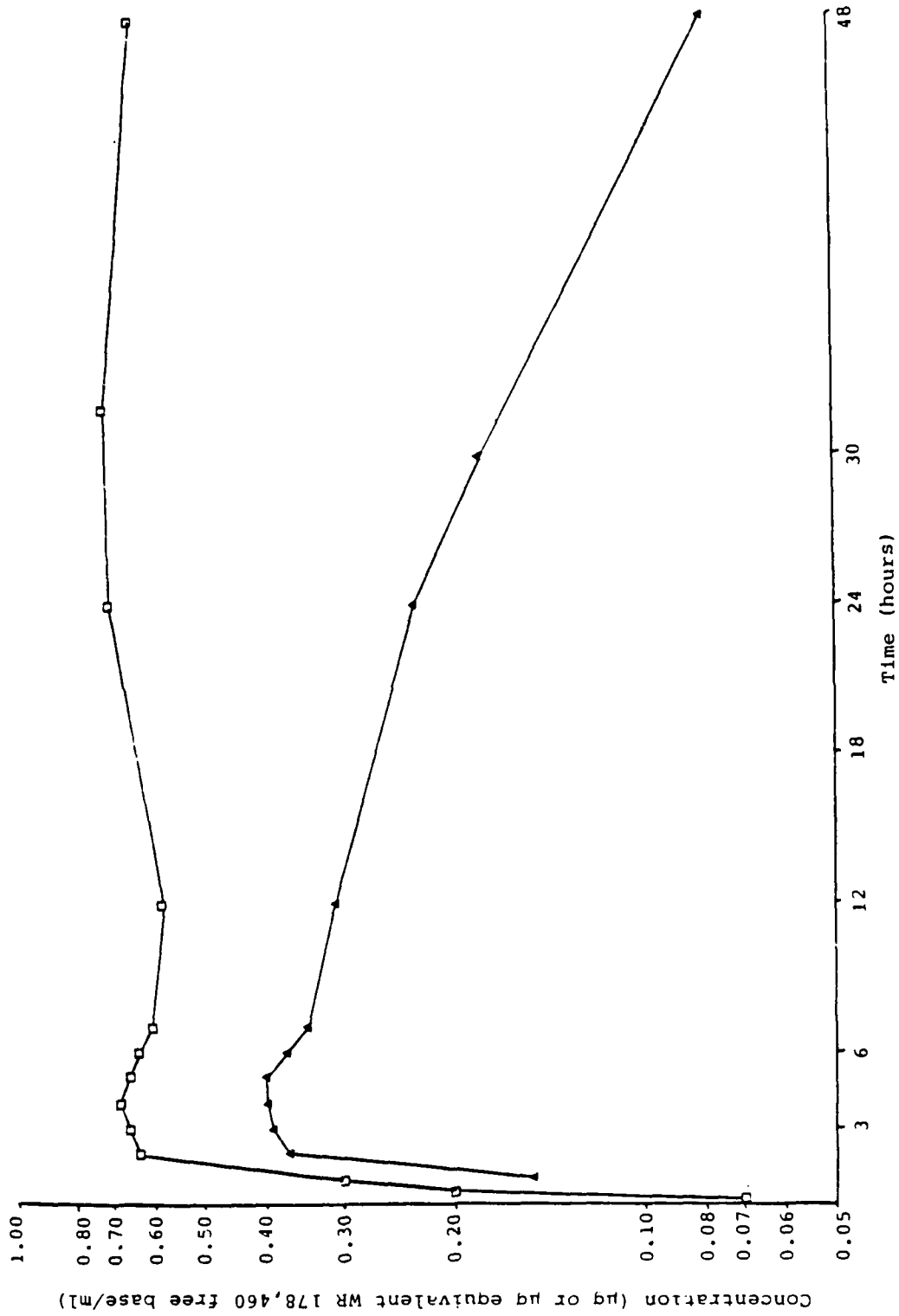


FIGURE 4

Observed points ( $\Delta$ ) and fitted curve for concentrations of total radioactivity in plasma after oral administration of  $^{14}\text{C}$ -WR 178,460.HCl to a beagle dog

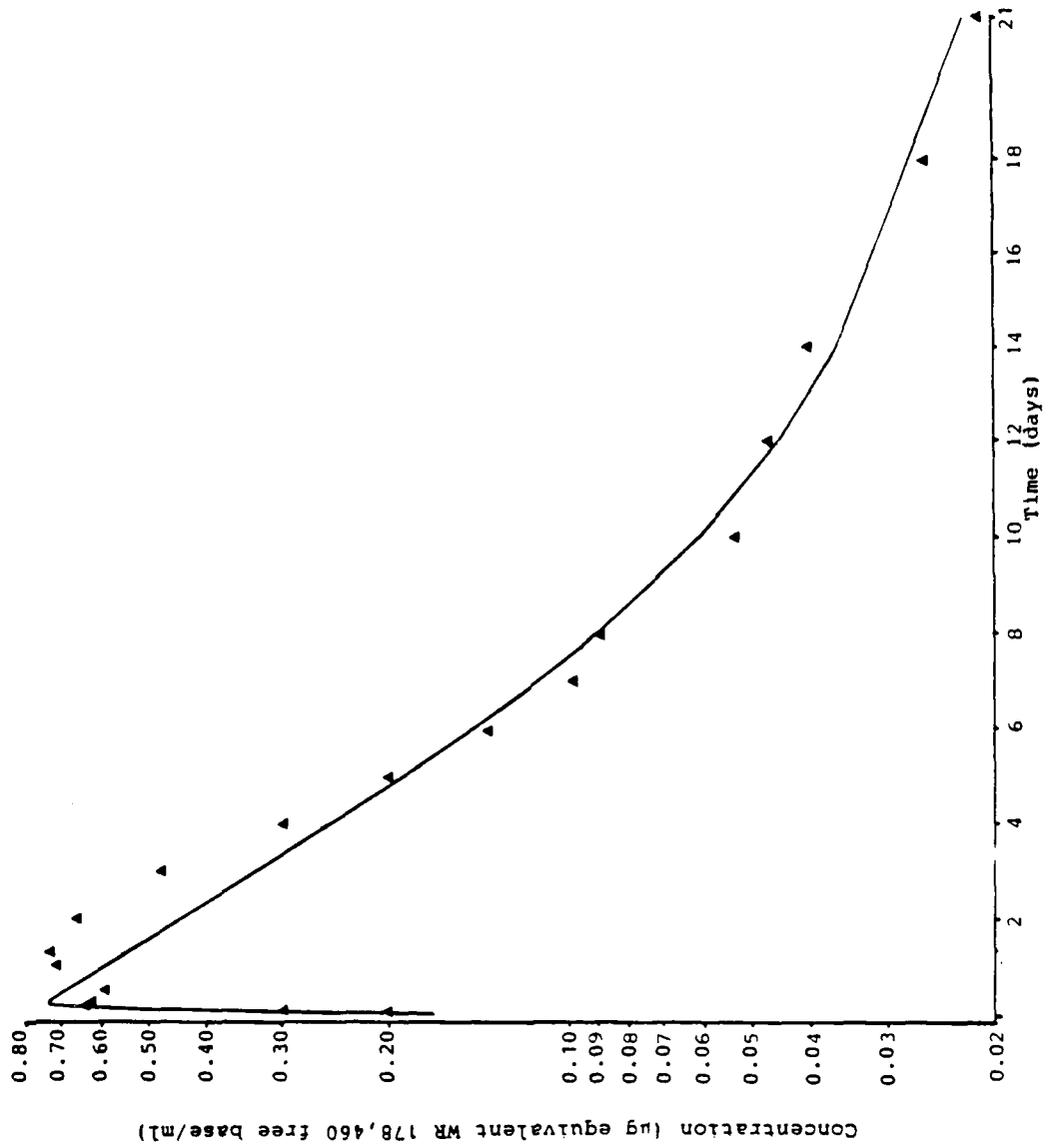
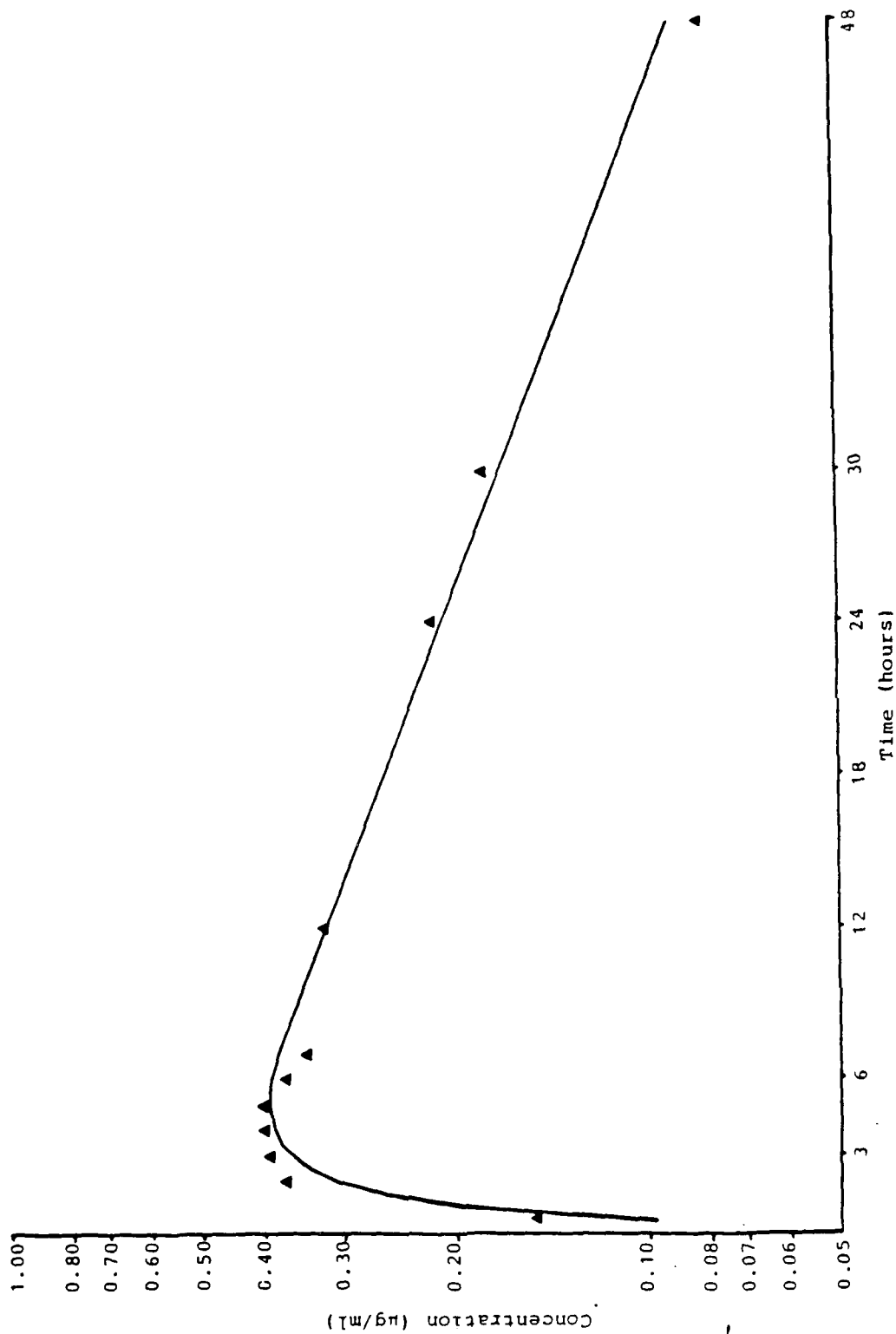


FIGURE 5  
Observed points ( $\Delta$ ) and fitted curve for concentrations of WR 178,460. in  
plasma after administration of  $^{14}$ C-WR 178,460.HCl to a beagle dog  
(time lag - 0.107 hours)



THE ABSORPTION, EXCRETION AND  
BIOTRANSFORMATION OF  $^{14}\text{C}$ -WR 158,122  
IN THE RHESUS MONKEY



## SUMMARY

1. The purpose of this study was to investigate the pharmacokinetics and metabolism of WR 158,122 a new potential anti-malarial drug in rhesus monkeys. Single 10 mg/kg oral doses of  $^{14}\text{C}$ -WR 158,122 have been administered in suspension in 2% aqueous sodium carboxymethylcellulose solution to both intact monkeys and monkeys with cannulated bile ducts. The effects of incorporating bile salt (2% sodium taurocholate) in solution in the dosing suspension have also been studied.
2. In the intact monkeys excretion of radioactivity in faeces of one male and one female animal accounted for 88.0% and 91.7% respectively of the dose during 6 days after oral administration of  $^{14}\text{C}$ -WR 158,122. Most of the drug (87.5% and 91.3% dose respectively) was excreted during the first 48 hours after dosing. Urinary excretion during the 6 days after dosing accounted for 1.07% (male) and 1.95% of dose (female).
3. After oral administration of  $^{14}\text{C}$ -WR 158,122 to monkeys with cannulated bile ducts excretion of radioactivity in the faeces accounted for 41.5% (male) and 71.2% of dose (female) during the 3 days after dosing. Excretion in urine accounted for 24.5% (male) and 6.42% of dose (female), and in bile accounted for 19.0% (male) and 3.07% (female) during 3 days after dosing. Most of the radioactivity was eliminated in bile, urine and faeces during the first 2 days after dosing.
4. In intact animals peak concentrations of radioactivity in plasma of 0.17  $\mu\text{g/ml}$  (male) and 0.22  $\mu\text{g/ml}$  (female) were reached at 5 hours. Concentrations declined to 0.12  $\mu\text{g/ml}$  (male) and 0.18  $\mu\text{g/ml}$  (female) at 6 hours, and rose again to 0.13  $\mu\text{g/ml}$  (male) and 0.37  $\mu\text{g/ml}$  (female peak level) at 24 hours, after which concentrations declined to 0.05  $\mu\text{g/ml}$  (male) and 0.14  $\mu\text{g/ml}$  (female) at 30 hours and were below the limit of accurate measurement (0.05  $\mu\text{g/ml}$ ) at 48 hours.

Concentrations of radioactivity in whole-blood were lower than corresponding plasma concentrations up to 6 hours after dosing. At 30 hours whole-blood concentrations were greater than those in plasma.

5. In animals with cannulated bile ducts, concentrations of radioactivity in plasma were greater than corresponding plasma concentrations in the intact animals. Peak concentrations of 3.42  $\mu\text{g/ml}$  (male) and 0.70  $\mu\text{g/ml}$  (female) were reached at 5 hours after dosing. There was no secondary peak at 24 hours when concentrations were 1.37  $\mu\text{g/ml}$  (male) and 0.29  $\mu\text{g/ml}$  (female). Concentrations continued to decline to 0.13  $\mu\text{g/ml}$  in the male at 54 hours and 0.04  $\mu\text{g/ml}$  in the female at 48 hours. Concentrations in the male at 72 hours and in the female at 54 hours were below the limit of accurate measurement (0.03  $\mu\text{g/ml}$ ).

Concentrations of radioactivity in whole-blood were lower than corresponding plasma concentrations at all times where concentrations were measurable.

6. Comparison of concentrations of radioactivity in plasma between monkeys receiving single oral doses of  $^{14}\text{C}$ -WR 158,122 with and without bile salt included in the dosing medium indicated that the addition of bile salt to the dose had no effect on the extent of absorption. Without bile salt incorporated in the dosing medium a peak of mean concentrations of 0.63  $\mu\text{g/ml}$  was reached at 4 hours after dosing, after which mean concentrations declined to 0.45  $\mu\text{g/ml}$ , 0.07  $\mu\text{g/ml}$  and 0.03  $\mu\text{g/ml}$  at 6, 24 and 30 hours respectively. With bile salt incorporated in the dosing medium a peak of mean concentrations of 0.33  $\mu\text{g/ml}$  was reached at 4 hours after dosing. Mean concentrations declined to 0.29  $\mu\text{g/ml}$ , 0.10  $\mu\text{g/ml}$  and 0.07  $\mu\text{g/ml}$  at 6, 24 and 30 hours respectively.

Mean areas under plasma radioactivity-time curves up to 24 hours after dosing were 0.67  $\text{g.h.ml}^{-1}.\text{mg}^{-1}.\text{kg}$  without bile salt and 0.43  $\text{g.h.ml}^{-1}.\text{mg}^{-1}.\text{kg}$  with bile salt added to the dosing medium.

7. Most of the radioactivity in urine was associated with two radioactive components corresponding to 14% and 50% of extracted urinary radioactivity. Most of the residual radioactivity was associated with 3 components which underwent partial hydrolysis on incubation with  $\beta$ -glucuronidase/arylsulphatase. Metabolite patterns in bile were similar to those in urine but different in proportion. The two major metabolites present in urine were also present in bile but mainly in a conjugated form. After enzymic hydrolysis these two metabolites accounted for 5% and 40% respectively of the extracted biliary radioactivity (compared with 5% or less for both components before hydrolysis). A third, more polar metabolite remained after enzymic hydrolysis and accounted for 10-20% of the biliary radioactivity. In extracts of faeces almost all the radioactivity was associated with unchanged WR 158,122 with small traces of the two main urinary metabolites.

TABLE 1

Excretion of radioactivity in the urine, faeces, cage debris and cage wash of rhesus monkeys after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as % dose/stated time interval

Time (h)	Monkey No.		Mean
	C502♂	B513♀	
Urine			
0- 6	0.00	0.67	0.34
6- 24	0.90	1.08	0.99
24- 48	0.13	0.14	0.14
48- 72	0.02	0.03	0.03
72- 96	0.01	0.01	0.01
96-120	0.00	0.01	0.01
120-144	0.01	0.01	0.01
Faeces			
0- 24	64.45	76.60	70.53
24- 48	23.05	14.70	18.88
48- 72	0.47	0.37	0.42
72- 96	0.01	0.02	0.02
96-120	ND	ND	0.00
120-144	0.00	0.00	0.00
Cage debris			
0- 24	0.28	1.78	1.03
24- 48	0.23	0.09	0.16
48- 72	0.06	0.01	0.04
72- 96	0.01	ND	0.01
96-120	0.01	ND	0.01
120-144	0.01	ND	0.01
Cage wash			
0- 6	0.01	0.06	0.04
6- 24	0.06	0.08	0.07
24- 48	0.01	0.02	0.02
48- 72	0.01	0.00	0.01
72- 96	0.00	ND	0.00
96-120	0.00	ND	0.00
96-120	0.17	0.12	0.15
120-144	0.01	ND	0.01
144-150	0.10	ND	0.05

ND Not detected (< 2 x background)

TABLE 2

Cumulative excretion of radioactivity in the urine, faeces, cage debris and cage washings of rhesus monkeys after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as % dose/stated time interval

Time (hours)	Monkey No.		Mean
	C502♂	B518♀	
Urine			
0- 6	0.00	0.67	0.34
0- 24	0.90	1.75	1.33
0- 48	1.03	1.89	1.46
0- 72	1.05	1.92	1.49
0- 96	1.06	1.93	1.50
0-120	1.06	1.94	1.50
0-144	1.07	1.95	1.51
Faeces			
0- 24	64.45	76.60	70.53
0- 48	87.50	91.30	89.40
0- 72	87.97	91.67	89.82
0- 96	87.98	91.69	89.84
0-120	87.98	91.69	89.84
0-144	87.98	91.69	89.84
Cage debris			
0- 24	0.28	1.78	1.03
0- 48	0.51	1.87	1.19
0- 72	0.57	1.88	1.23
0- 96	0.58	1.88	1.23
0-120	0.59	1.88	1.24
0-144	0.60	1.88	1.24
Cage washings			
0- 6	0.01	0.06	0.04
0- 24	0.07	0.14	0.11
0- 48	0.08	0.16	0.12
0- 72	0.09	0.16	0.13
0- 96	0.09	0.16	0.13
0-120	0.09	0.16	0.13
0-120	0.26	0.28	0.27
0-144	0.27	0.28	0.28
0-150	0.37	0.28	0.33

TABLE 3

Excretion of radioactivity in the bile, urine and faeces of  
 rhesus monkeys with cannulated bile ducts after single oral doses of  
<sup>14</sup>C-WR 158,122

Results are expressed as % dose/stated time interval

Time (hours)	Monkey No.		Mean
	C502 <del>8</del>	B519 <del>8</del>	
Bile			
0- 2	0.09	0.08	0.09
2- 4	1.32	0.28	0.80
4- 6	2.13	0.27	1.20
6- 8	1.96	0.31	1.14
8-24	9.43	1.52	5.48
24-30	1.77	0.31	1.04
30-48	1.85	0.25	1.05
48-54	0.25	0.03	0.14
54-72	0.20	0.02	0.11
Urine			
0- 2	0.01	0.16	0.09
2- 4	2.91	0.39	1.65
4- 6	0.16	0.47	0.32
6- 8	NS	0.12	0.06
8-24	12.29	4.16	8.23
24-30	1.99	0.50	1.25
30-48	4.51	0.43	2.47
48-54	1.48	0.08	0.78
54-72	1.11	0.11	0.61
Faeces			
0-24	15.82	51.73	33.78
24-48	23.55	18.74	21.15
48-72	2.17	0.70	1.44

NS No sample

TABLE 4

Cumulative excretion of radioactivity in the bile, urine and faeces of rhesus monkeys with cannulated bile ducts after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as % dose/stated time interval

Time (hours)	Monkey No.		Mean
	C502s	B518s	
Bile			
0- 2	0.09	0.08	0.09
0- 4	1.41	0.36	0.89
0- 6	3.54	0.63	2.09
0- 8	5.50	0.94	3.22
0-24	14.93	2.46	8.70
0-30	16.70	2.77	9.74
0-48	18.55	3.02	10.79
0-54	18.80	3.05	10.93
0-72	19.00	3.07	11.04
Urine			
0- 2	0.01	0.16	0.09
0- 4	2.92	0.55	1.74
0- 6	3.08	1.02	2.05
0- 8	3.08	1.14	2.11
0-24	15.37	5.30	10.34
0-30	17.36	5.80	11.58
0-48	21.87	6.23	14.05
0-54	23.35	6.31	14.83
0-72	24.46	6.42	15.44
Faeces			
0-24	15.82	51.73	33.78
0-48	39.37	70.47	54.92
0-72	41.54	71.17	56.36

TABLE 5

Concentrations of radioactivity in the plasma of rhesus monkeys after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as  $\mu\text{g}$  equivalents/ml

Time (hours)	Monkey No.		Mean
	C5028	B5188	
0	ND	ND	0.00
0.5	ND	ND	0.00
1	ND	ND	0.00
2	0.05	ND	0.03
3	0.08	0.09	0.09
4	0.16	0.20	0.18
5	0.17	0.22	0.20
6	0.12	0.18	0.15
24	0.13	0.37	0.25
30	0.05	0.14	0.10
48	ND	ND	0.00

ND Not detected ( $<0.05 \mu\text{g/ml}$ )

ND assumed as zero in calculation of the means



TABLE 6

Concentrations of radioactivity in the plasma of rhesus monkeys after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as % dose/litre

Time (hours)	Monkey No.		Mean
	C5028	B5188	
0	ND	ND	0.00
0.5	ND	ND	0.00
1	ND	ND	0.00
2	0.08	ND	0.04
3	0.13	0.23	0.18
4	0.28	0.53	0.41
5	0.29	0.58	0.44
6	0.21	0.47	0.34
24	0.22	0.95	0.59
30	0.08	0.36	0.22
48	ND	ND	0.00

ND Not detected (<0.08 % dose/litre)

ND assumed as zero in calculation of the means

TABLE 7

Concentrations of radioactivity in the whole-blood of rhesus monkeys  
after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as  $\mu\text{g}$  equivalents/g

Time (hours)	Monkey No.		Mean
	C5026	B5198	
0	ND	ND	0.00
0.5	ND	ND	0.00
1	ND	ND	0.00
2	ND	ND	0.00
3	ND	ND	0.00
4	0.09	0.12	0.11
5	0.10	0.13	0.12
6	ND	0.11	0.06
24	0.12	0.30	0.21
30	0.21	0.24	0.23
48	ND	ND	0.00

ND Not detected ( $<0.08 \mu\text{g/g}$ )

ND assumed as zero in calculation  
of the means

TABLE 8

Concentrations of radioactivity in the whole-blood of rhesus monkeys  
after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as % dose/kg

Time (hours)	Monkey No.		Mean
	C5028	B5189	
0	ND	ND	0.00
0.5	ND	ND	0.00
1	ND	ND	0.00
2	ND	ND	0.00
3	ND	ND	0.00
4	0.15	0.30	0.23
5	0.17	0.34	0.26
6	ND	0.28	0.14
24	0.20	0.78	0.49
30	0.36	0.63	0.50
48	ND	ND	0.00

ND Not detected (<0.16 % dose/kg)

ND assumed as zero in calculation  
of the means

TABLE 9

Concentrations of radioactivity in the plasma of bile duct cannulated rhesus monkeys after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as  $\mu\text{g}$  equivalents/ml

Time (hours)	Monkey No.		Mean
	C502♂	B518♀	
0	ND	ND	0.00
0.5	0.20	0.09	0.15
1	0.74	0.23	0.49
2	1.53	0.40	0.97
3	2.45	0.56	1.51
4	3.07	0.66	1.87
5	3.42	0.70	2.06
6	3.27	0.66	1.97
24	1.37	0.29	0.83
30	0.75	0.15	0.45
48	0.21	0.04	0.13
54	0.13	ND	0.07
72	ND	ND	0.00

ND Not detected ( $<0.03 \mu\text{g/ml}$ )

ND assumed as zero in calculation of the means

TABLE 10

Concentrations of radioactivity in the plasma of bile duct cannulated rhesus monkeys after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as % dose/litre

Time (hours)	Monkey No.		Mean
	C5024	B5198	
0	ND	ND	0.00
0.5	0.34	0.24	0.29
1	1.28	0.61	0.95
2	2.64	1.06	1.85
3	4.23	1.49	2.86
4	5.28	1.75	3.52
5	5.90	1.88	3.89
6	5.63	1.75	3.69
24	2.35	0.77	1.56
30	1.30	0.41	0.86
48	0.37	0.10	0.24
54	0.22	ND	0.11
72	ND	ND	0.00

ND Not detected (<0.06 % dose/litre)

ND assumed as zero in calculation of the means

TABLE 11

Concentrations of radioactivity in the whole-blood of bile duct cannulated rhesus monkeys after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as  $\mu\text{g}$  equivalents/g

Time (hours)	Monkey No.		Mean
	C502♂	B518♀	
0	ND	ND	0.00
0.5	0.12	ND	0.06
1	0.46	0.16	0.31
2	0.96	0.25	0.61
3	1.54	0.36	0.95
4	1.99	0.45	1.22
5	2.23	0.47	1.35
6	2.14	0.45	1.30
24	0.85	0.18	0.52
30	0.49	0.09	0.29
48	0.15	ND	0.08
54	NS	ND	0.00
72	ND	ND	0.00

ND Not detected ( $<0.07 \mu\text{g/g}$ )

NS No sample

ND assumed as zero in calculation of the means

TABLE 12

Concentrations of radioactivity in the whole-blood of bile duct cannulated rhesus monkeys after single oral doses of  $^{14}\text{C}$ -WR 158,122

Results are expressed as % dose/kg

Time (hours)	Monkey No.		Mean
	C502 <del>8</del>	B519 <del>8</del>	
0	ND	ND	0.00
0.5	0.21	ND	0.11
1	0.78	0.41	0.60
2	1.65	0.66	1.16
3	2.66	0.97	1.82
4	3.42	1.19	2.31
5	3.84	1.26	2.55
6	3.69	1.19	2.44
24	1.46	0.49	0.98
30	0.84	0.25	0.55
48	0.26	ND	0.13
54	NS	ND	0.00
72	ND	ND	0.00

ND Not detected (<0.14 % dose/kg)

NS No sample

ND assumed as zero in calculation of the means

TABLE 13

Concentrations of radioactivity in the plasma of rhesus monkeys after oral doses of  $^{14}\text{C}$ -WR 158,122 with and without incorporation of bile salt in the dose suspension

Results are expressed as  $\mu\text{g}$  equivalents/ml

(a) Dose suspended in carboxymethylcellulose solution

Time (hours)	Monkey No.			Mean	SD ±
	C498♀	C505♀	343♀		
0	ND	ND	ND	0.00	0.00
0.5	ND	ND	ND	0.00	0.00
1	0.06	0.08	0.05	0.06	0.02
2	0.23	0.50	0.24	0.32	0.15
3	0.21	0.69	0.66	0.52	0.27
4	0.20	0.74	0.95	0.63	0.39
5	0.17	0.61	0.81	0.53	0.33
6	0.15	0.50	0.69	0.45	0.27
24	0.07	0.08	0.05	0.07	0.02
30	0.05	0.05	ND	0.03	0.03
48	ND	ND	ND	0.00	0.00
72	ND	ND	ND	0.00	0.00

(b) Dose suspended in carboxymethylcellulose solution incorporating bile salt

Time (hours)	Monkey No.			Mean	SD ±
	C498♀	C505♀	343♀		
0	ND	ND	ND	0.00	0.00
0.5	ND	ND	ND	0.00	0.00
1	0.05	ND	ND	0.02	0.03
2	0.09	0.09	0.33	0.17	0.14
3	0.09	0.11	0.48	0.23	0.22
4	0.10	0.10	0.80	0.33	0.40
5	0.09	0.08	0.76	0.31	0.39
6	0.09	0.09	0.68	0.29	0.34
24	0.06	0.13	0.11	0.10	0.04
30	0.05	0.08	0.07	0.07	0.02
48	ND	ND	ND	0.00	0.00

ND Not detected ( $<0.04 \mu\text{g/ml}$ )

SD Standard deviation

ND assumed as zero in calculation of the means



TABLE 14

Concentrations of radioactivity in the plasma of rhesus monkeys after oral doses of  $^{14}\text{C}$ -WR 158,122 with and without incorporation of bile salt in the dose suspension

Results are expressed as % dose/litre

(a) Dose suspended in carboxymethylcellulose solution

Time (hours)	Monkey No.			Mean	SD ±
	C498%	C505%	343%		
0	ND	ND	ND	0.00	0.00
0.5	ND	ND	ND	0.00	0.00
1	0.10	0.13	0.06	0.10	0.04
2	0.37	0.83	0.28	0.49	0.30
3	0.34	1.15	0.78	0.76	0.41
4	0.32	1.23	1.12	0.89	0.50
5	0.28	1.02	0.97	0.76	0.41
6	0.25	0.82	0.82	0.63	0.33
24	0.12	0.13	0.06	0.10	0.04
30	0.09	0.08	ND	0.06	0.05
48	ND	ND	ND	0.00	0.00
72	ND	ND	ND	0.00	0.00

(b) Dose suspended in carboxymethylcellulose solution incorporating bile salt

Time (hours)	Monkey No.			Mean	SD ±
	C498%	C505%	343%		
0	ND	ND	ND	0.00	0.00
0.5	ND	ND	ND	0.00	0.00
1	0.08	ND	ND	0.03	0.05
2	0.14	0.15	0.41	0.23	0.15
3	0.15	0.19	0.59	0.31	0.24
4	0.16	0.17	1.00	0.44	0.48
5	0.14	0.12	0.95	0.40	0.47
6	0.14	0.15	0.84	0.38	0.40
24	0.10	0.22	0.14	0.15	0.06
30	0.08	0.13	0.08	0.10	0.03
48	ND	ND	ND	0.00	0.00

ND Not detected (<0.06 % dose/litre)

SD Standard deviation

ND assumed as zero in calculation of the means

TABLE 15

Areas under plasma radioactivity concentration time curves up to 24 hours after single oral doses of  $^{14}\text{C}$ -WR 158,122 to rhesus monkeys

Animal No.	Dose level	Area ( $\mu\text{g.h.ml}^{-1}$ )	Area/dose level ( $\mu\text{g.h.ml}^{-1}.\text{mg}^{-1}.\text{kg}$ )
<u>Intact animals</u>			
C502s	10.3	2.77	0.27
B518s	9.8	5.55	0.57
<u>Bile duct cannulated animals</u>			
C502s	10.1	54.52	5.40
B518s	9.5	11.42	1.20
<u>Intact animals (No bile salt in dosing medium)</u>			
C498s	10.0	2.91	0.29
C505s	10.0	8.07	0.81
343s	10.5	9.70	0.92
Mean $\pm$ SD			0.67 $\pm$ 0.34
<u>Intact animals (Bile salt added to dosing medium)</u>			
C498s	9.9	1.80	0.18
C505s	10.5	2.41	0.23
343s	11.0	9.82	0.89
Mean $\pm$ SD			0.43 $\pm$ 0.40

SD Standard deviation

TABLE 16

Observed (O) and expected (E) values for fitted models for total radioactivity concentrations in plasma from intact and bile duct cannulated monkeys

Results are expressed as dpm/ml

Time (hours)	Intact monkeys				Bile duct cannulated monkeys			
	C5028		B5189		C5028		B5189	
	O	E	O	E	O	E	O	E
0.5	-	-	-	-	620	616	280	286
1	-	-	-	-	2328	2373	713	665
2	155	149	-	-	4793	5221	1243	1265
3	235	278	278	283	7675	7322	175	1693
4	513	365	638	482	9588	8823	2053	1986
5	538	422	700	636	10705	9843	2200	2173
6	388	457	563	754	10220	10481	2053	2278
24	405	253	1125	568	4273	4493	905	854
30	150	184	435	374	2358	2731	483	509
48	-	-	-	-	670	545	115	99
54	-	-	-	-	395	313	-	-
Model parameters								
$\tau$ (h)	1.1635		1.8874		0.3399		0.1661	
$\lambda_1$ ( $h^{-1}$ )	0.0538		0.1093		0.0935		0.1986	
$\lambda_2$ ( $h^{-1}$ )	0.2898		0.1093		0.1754		0.0930	
A (dpm/ml)	871.8225		7561224		48014.8789		-8514.8516	
$h_1$	12.9		6.34		7.41		3.49	
$h_2$	2.39		6.34		3.95		7.45	

TABLE 17

Observed (O) and expected (E) values for fitted models for total radioactivity concentrations in plasma from monkeys after oral doses with and without incorporation of bile salt in the dosing suspension

Results are expressed as dpm/ml

Time (hours)	Bile salt omitted						Bile salt added					
	C498†		C505†		343†		C498†		C505†		343†	
	O	E	O	E	O	E	O	E	O	E	O	E
1	200	200	253	252	168	161	170	171	-	-	-	-
2	743	690	1653	1780	793	1132	295	285	308	180	1093	1054
3	678	665	2290	2039	2180	1714	310	318	375	246	1568	1868
4	653	631	2450	1970	3118	2022	335	323	340	300	2653	2124
5	558	598	2023	1826	2688	2141	385	318	250	343	2515	2134
6	500	568	1640	1672	2285	2134	293	309	305	377	2228	2040
24	235	219	255	315	178	183	203	168	438	303	370	426
30	173	159	155	181	-	-	175	136	260	222	218	249
Model parameters												
$\tau$ (h)	0.9183		0.9184		0.8721		0.3736		0.0000		1.3814	
$\lambda_1$ ( $h^{-1}$ )	0.0530		1.3137		0.2197		0.0341		0.0873		0.0894	
$\lambda_2$ ( $h^{-1}$ )	3.9120		0.0928		0.2207		1.0297		0.0910		0.7763	
A (dpm/ml)	742.7436		-2684.9568		1282217.		375.9407		2960.9922		3217.4402	
$h_1$	13.1		0.53		3.15		20.3		7.94		7.75	
$h_2$	0.18		7.47		3.14		0.67		7.62		0.89	

TABLE 18

Proportions of radioactive components in methanol extracts of urine from monkeys with cannulated bile ducts after oral doses of  $^{14}\text{C}$ -WR 158,122  
 Solvent system: Chloroform:methanol:water:formic acid (80:25:3:3, v/v)

Results are expressed as a proportion (%) of total radioactivity in each chromatogram

Animal No.	C502d															B518g		
	2-4 hours			8-24 hours			24-30 hours			30-48 hours			48-54 hours			8-24 hours		
	U	C	E	U	C	E	U	C	E	U	C	E	U	C	E	U	C	E
0.00	<0.9	<1.2	1.2	1.4	2.8	3.3	1.8	2.4	2.0	1.9	2.0	2.1	3.5	3.6	5.2	2.9	3.9	3.3
0.04	3.8	3.0	1.2	4.3	4.0	2.5	3.5	4.0	2.7	2.6	3.2	2.5	11.3	13.5	8.2	27.0	34.4	5.1
0.07	9.3	9.1	3.2	6.0	7.3	4.1	5.9	7.0	4.6	7.2	4.3	4.6	29.4	24.4	15.1	18.6	20.2	5.6
0.10	7.9	8.8	5.5	13.0	14.0	3.1	25.1	24.1	4.3	28.1	31.1	6.5	10.7	12.7	11.7	46.5	37.8	22.7
0.20	17.0	17.2	21.1	14.8	15.6	17.8	12.0	11.5	16.5	10.8	9.6	16.6	41.3	40.3	49.4	18.6	20.2	51.7
0.24	56.7	56.4	60.9	56.3	52.3	52.7	48.0	46.9	58.9	45.7	45.5	58.1	2.8	-	2.2	46.5	37.8	51.7
0.41	-	-	<0.9	-	-	2.3	-	-	3.0	-	-	2.8	-	-	2.2	-	-	4.8
0.45	-	-	1.0	-	-	2.6	-	-	2.3	-	-	2.5	-	-	1.2	-	-	3.2
0.49	-	-	1.3	-	-	1.6	-	-	-	-	-	-	-	-	1.2	-	-	<1.0
0.52	-	-	<0.9	-	-	1.1	-	-	-	-	-	-	-	-	-	-	-	-
Others	4.5	4.6	3.5	4.1	4.0	9.0	3.8	4.1	5.7	3.7	4.4	4.4	3.8	5.4	5.8	5.0	3.7	2.8

U Untreated urine

C Urine incubated at pH 5, 37°C, ca. 17 hours

E Enzyme-treated urine (Incubated at pH 5, 37°C. 17 hours in the presence of  $\beta$ -glucuronidase/aryl sulphatase)

TABLE 19

Proportions of radioactive components in methanol extracts of urine from monkeys with cannulated bile ducts after oral doses of  $^{14}\text{C}$ -WR 158,122  
 Solvent system: Chloroform:methanol:water:formic acid (80:25:3:3, v/v)

Results are expressed as % dose\*

Animal No.	C5023															B5189				
R <sub>f</sub> value	2-4 hours			8-24 hours			24-30 hours			30-48 hours			48-54 hours			8-24 hours				
	U	C	E	U	C	E	U	C	E	U	C	E	U	C	E	U	C	E		
0.00	<0.03	<0.03	0.03	0.17	0.34	0.35	0.04	0.05	0.04	0.08	0.09	0.08	0.05	0.05	0.07	0.12	0.16	0.12	E	
0.04	0.11	0.09	0.03	0.52	0.49	0.28	0.07	0.08	0.05	0.12	0.14	0.10	0.16	0.20	0.11	1.1	1.4	0.18	E	
0.07	0.27	0.26	0.09	0.73	0.89	0.45	0.12	0.14	0.08	0.32	0.19	0.18	0.43	0.36	0.20	0.76	0.84	0.20	E	
0.10	0.23	0.26	0.15	1.6	1.7	0.34	0.49	0.47	0.08	1.3	1.4	0.26	0.16	0.19	0.15	1.9	1.6	1.8	E	
0.20	0.49	0.50	0.58	1.2	1.9	2.0	0.24	0.23	0.30	0.48	0.43	0.67	0.60	0.59	0.65	-	-	0.17	E	
0.24	1.6	1.6	1.7	6.9	6.4	5.8	0.95	0.92	1.1	2.0	2.0	2.3	0.60	0.59	0.65	1.9	1.6	1.8	E	
0.41	-	-	<0.02	-	-	0.25	-	-	0.05	-	-	0.11	-	-	0.03	-	-	0.17	E	
0.45	-	-	0.03	-	-	0.29	-	-	0.04	-	-	0.10	-	-	0.02	-	-	0.11	E	
0.49	-	-	0.04	-	-	0.18	-	-	-	-	-	-	-	-	0.02	-	-	<0.04	E	
0.52	-	-	<0.02	-	-	0.12	-	-	-	-	-	-	-	-	-	-	-	-	E	
Others	0.13	0.13	0.10	0.50	0.49	1.0	0.07	0.08	0.10	0.17	0.20	0.18	0.06	0.08	0.08	0.20	0.15	0.10	E	

U Untreated urine

C Urine incubated at pH 5, 37°C, ca. 17 hours

E Enzyme-treated urine (Incubated at pH 5, 37°C. 17 hours in the presence of  $\beta$ -glucuronidase/aryl sulphatase)

\* Calculated as proportion (%) radioactivity in chromatogram x % dose in sample x  $\frac{\text{proportion extracted in methanol}}{100}$

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TABLE 20

Proportions of radioactive components in methanol extracts of bile from monkeys with cannulated bile ducts after oral doses of  $^{14}\text{C}$ -WR 158,122

Solvent system: Chloroform:methanol:water:formic acid (80:25:3:3, v/v)

Results are expressed as a proportion (%) of the total radioactivity in each chromatogram

Animal No.	C502f										B518g					
	0-8 hours			8-24 hours			24-48 hours			E	0-8 hours			8-24 hours		
R <sub>f</sub> value	U	C	E	U	C	E	U	C	E		U	C	E	U	C	E
0	2.6	2.4	<1.0	3.1	3.2	1.6	<4.5	<4.9	<5.0		<4.2	<3.9	<4.0	<3.9	<3.6	<3.7
0.02	1.3	1.6	<1.0	1.4	1.5	<1.1	<4.5	<4.9	<5.0		<4.2	<3.9	<4.0	<3.9	<3.6	<3.7
0.03	3.6	3.1	1.0	4.2	3.4	1.4	<4.5	<4.9	<5.0		<4.2	<3.9	<4.0	4.1	<3.6	<3.7
0.04	29.8	23.9	3.7	28.0	26.8	6.4	24.3	27.1	8.0		23.3	20.5	<4.0	28.9	24.9	<3.7
0.07	6.9	10.3	1.1	7.2	7.1	<1.1	9.7	10.6	<5.0		26.2	26.8	<4.0	16.4	17.5	<3.7
0.10	40.5	42.4	20.9	41.4	45.7	8.1	53.7	46.6	11.5		32.1	34.7	19.2	36.2	36.9	11.0
0.20	2.0	2.4	5.0	<1.0	1.3	4.9	<4.5	<4.9	5.6		<4.2	<3.9	4.3	<3.9	<3.6	5.7
0.24	5.2	5.3	33.5	4.0	4.1	37.4	<4.5	<4.9	43.7		<4.2	<3.9	44.0	<3.9	3.8	42.2
0.41	<1.0	<1.0	7.2	<1.0	<1.1	10.8	<4.5	<4.9	9.5		<4.2	<3.9	10.1	<3.9	<3.6	8.6
0.45	<1.0	<1.0	<6.2	<1.0	<1.1	2.8	<4.5	<4.9	<5.0		<4.2	<3.9	<9.2	<3.9	<3.6	<9.3
0.49	<1.0	<1.0	<1.0	<1.0	<1.1	2.0	<4.5	<4.9	<5.0		<4.2	<3.9	<4.0	<3.9	<3.6	<3.7
0.52	<1.0	1.1	2.3	<1.0	<1.1	1.7	<4.5	<4.9	<5.0		<4.2	<3.9	<4.0	<3.9	<3.6	<3.7
Others	7.1	7.2	17.6	9.1	5.9	21.2	<4.5	<4.9	16.2		5.9	5.2	8.3	6.6	8.6	17.0

U Untreated bile

C Bile incubated at pH 5, 37°C, ca. 17 hours

E Enzyme-treated bile (Incubated at pH 5, 37°C, 17 hours in the presence of  $\beta$ -glucuronidase/aryl sulphatase)

TABLE 21

Proportions of radioactive components in methanol extracts of bile from rhesus monkeys with cannulated bile ducts after oral doses of  $^{14}\text{C}$ -WR 158,122  
 Solvent system: Chloroform:methanol:water:formic acid (80:25:3:3, v/v)

Results are expressed as % dose\*

Animal No.	C502♂												C518♀											
	0-8 hours				8-24 hours				24-48 hours				0-8 hours				8-24 hours							
	U	C	E		U	C	E		U	C	E		U	C	E		U	C	E					
0	0.14	0.13	<0.05		0.28	0.29	0.13		<0.15	<0.16	<0.14		<0.04	<0.03	<0.03		<0.06	<0.05	<0.03					
0.02	0.07	0.08	<0.05		0.13	0.13	<0.09		<0.15	<0.16	<0.14		<0.04	<0.03	<0.03		<0.06	<0.05	<0.03					
0.03	0.19	0.16	0.05		0.38	0.30	0.11		<0.15	<0.16	<0.14		<0.04	<0.03	<0.03		0.06	<0.05	<0.03					
0.04	1.6	1.2	0.18		2.6	2.4	0.51		0.82	0.88	0.23		0.21	0.18	<0.03		0.42	0.35	<0.03					
0.07	0.37	0.54	0.05		0.66	0.64	<0.09		0.33	0.35	<0.14		0.24	0.24	<0.03		0.24	0.24	<0.03					
0.10	2.2	2.2	1.0		3.8	4.1	0.64		1.8	1.5	0.32		0.29	0.31	0.17		0.53	0.52	0.15					
0.20	0.11	0.13	0.24		<0.09	0.12	0.39		<0.15	<0.16	0.16		<0.04	<0.03	0.04		<0.06	<0.05	0.08					
0.24	0.28	0.28	1.6		0.37	0.37	3.0		<0.15	<0.16	1.2		<0.04	<0.03	0.38		<0.06	0.05	0.56					
0.41	<0.05	<0.05	0.35		<0.09	<0.10	0.86		<0.15	<0.16	0.27		<0.04	<0.03	0.09		<0.06	<0.05	0.12					
0.45	<0.05	<0.05	0.30		<0.09	<0.10	0.22		<0.15	<0.16	<0.14		<0.04	<0.03	<0.08		<0.06	<0.05	<0.12					
0.49	<0.05	<0.05	0.11		<0.09	<0.10	0.16		<0.15	<0.16	<0.14		<0.04	<0.03	<0.03		<0.06	<0.05	<0.05					
0.52	<0.05	0.06	0.11		<0.09	<0.10	0.13		<0.15	<0.16	<0.14		<0.04	<0.03	<0.03		<0.06	<0.05	<0.05					
Others	0.38	0.38	0.86		0.83	0.53	1.7		<0.15	<0.16	0.46		0.05	0.05	0.07		0.10	0.12	0.23					

U Untreated bile

C Bile incubated at pH 5, 37°C, ca. 17 hours

E Enzyme-treated bile (incubated at pH 5, 37°C, 17 hours in the presence of  $\beta$ -glucuronidase/aryl sulphatase

\* Calculated as proportion (%) radioactivity in chromatogram x % dose in sample x  $\frac{100}{\text{proportion extracted in methanol}}$

HRC/WRI 3/84457



TABLE 22

Proportions of radioactivity in extracts of faeces of monkeys with cannulated bile ducts after oral doses of  $^{14}\text{C}$ -WR 158,122  
Solvent system: chloroform:methanol:water:formic acid (80:25:3:3, v/v)

(a) Results are expressed as a proportion (%) of the total radioactivity in each chromatogram

Animal No.	C502♂		B518♀	
R <sub>f</sub> value	0-24 hours	24-48 hours	0-24 hours	24-48 hours
0	0.7	<0.8	<0.8	<0.7
0.20	<0.7	1.6	<0.8	<0.7
0.24	2.4	8.3	<0.8	2.1
0.59+	88.8	83.0	96.8	93.5
0.63	1.4	0.9	0.9	1.2
0.72	-	1.2	-	-
Others	6.2	4.3	1.7	3.1

(b) Results are expressed as % dose\*

Animal No.	C502♂		B518♀	
R <sub>f</sub> value	0-24 hours	24-48 hours	0-24 hours	24-48 hours
0	0.11	<0.19	<0.40	<0.12
0.20	<0.11	0.37	<0.40	<0.12
0.24	0.38	1.9	<0.40	0.36
0.59+	14	19	48	16
0.63	0.22	0.21	0.45	0.21
0.72	-	0.28	-	-
Others	0.98	1.0	0.84	0.53

\* Calculated as  $\frac{\text{proportion (\% of radioactivity in chromatogram)} \times \text{\% dose in sample} \times \text{proportion extracted}}{100}$

+ Radioactive zone corresponding to WR 158,122

FIGURE 1

Cumulative excretion of radioactivity in urine and faeces of monkeys  
after oral doses of  $^{14}\text{C}$ -WR 158,122

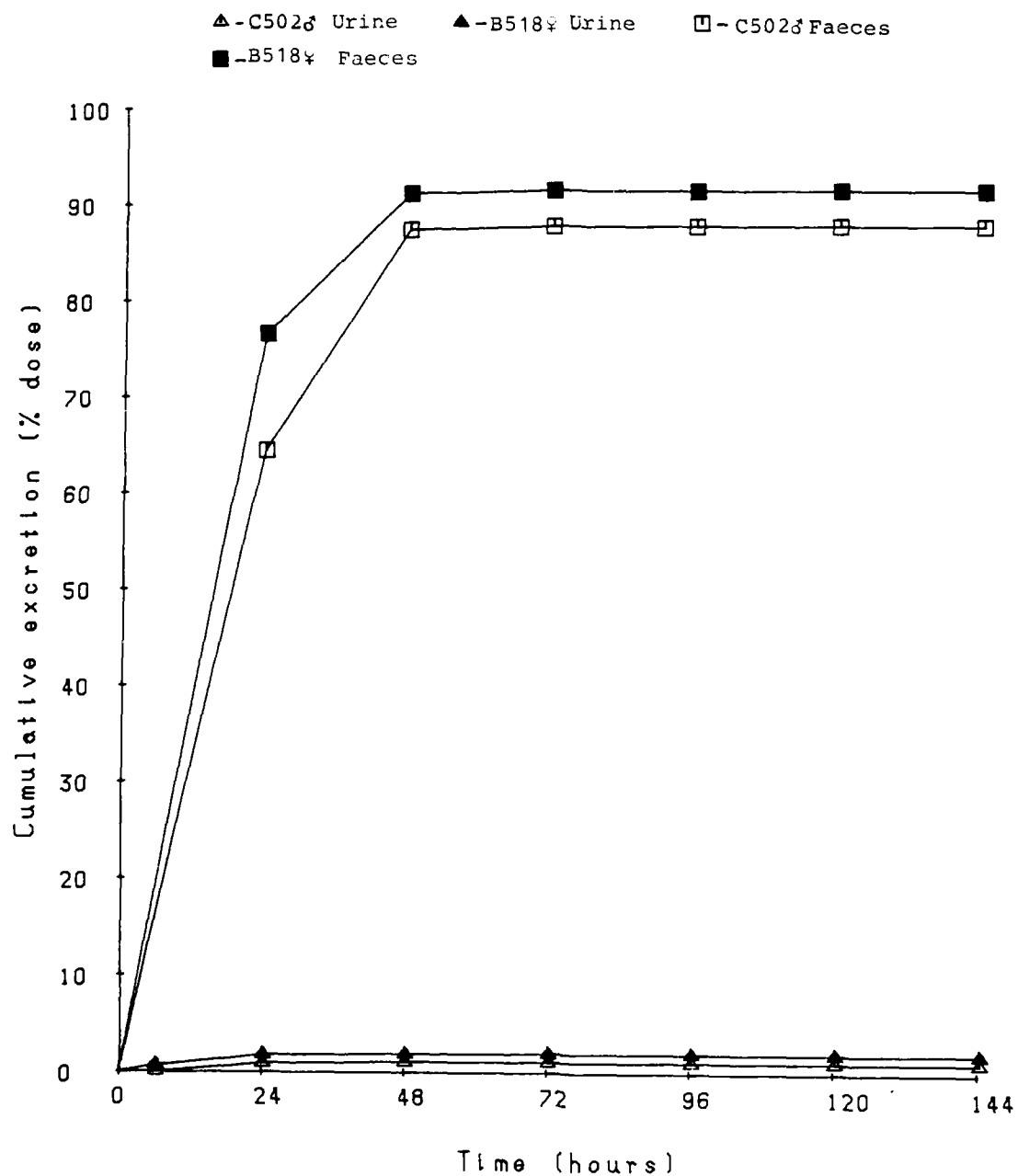


FIGURE 2

Cumulative excretion of radioactivity in urine, bile and faeces of monkeys with cannulated bile ducts after oral doses of  $^{14}\text{C}$ -WR 158,122

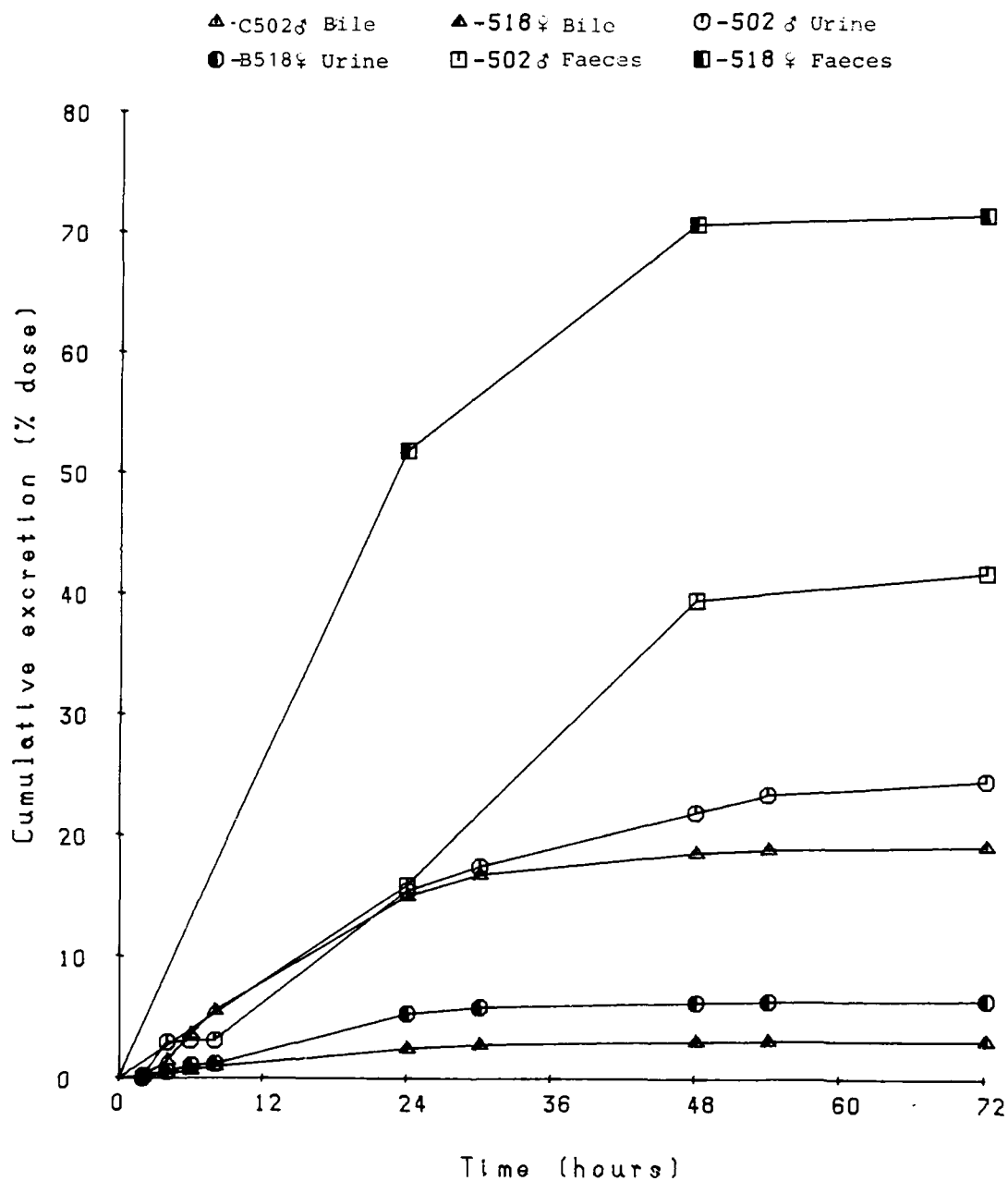
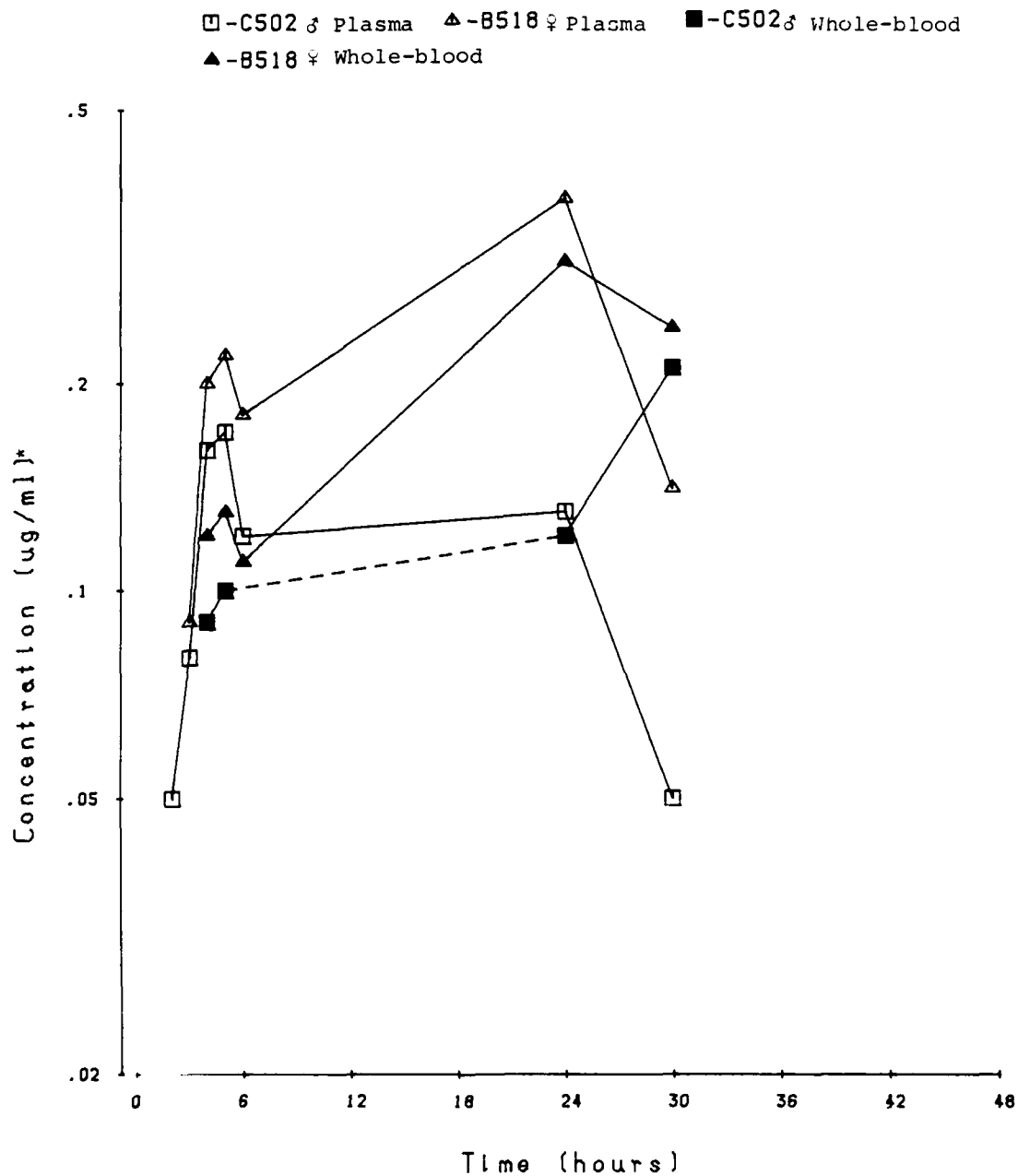


FIGURE 3

Concentrations of radioactivity in the plasma and whole-blood of monkeys  
after oral doses of  $^{14}\text{C}$ -WR 158,122



\* Concentrations of whole-blood are measured in  $\mu\text{g/g}$

FIGURE 4

Concentrations of radioactivity in the plasma of monkeys with cannulated  
bile ducts and in the plasma of intact monkeys after oral doses of  
<sup>14</sup>C-WR 158,122

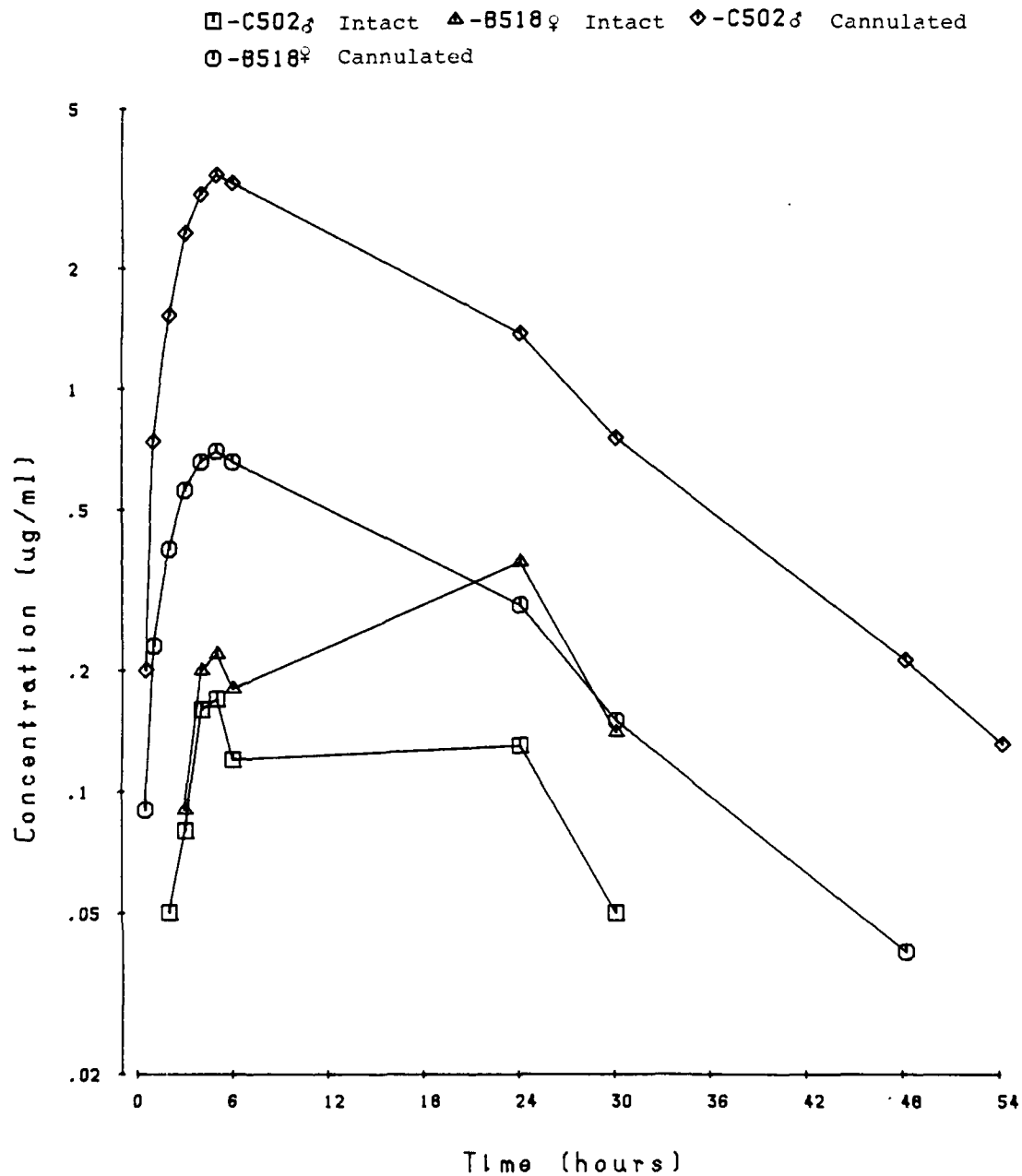
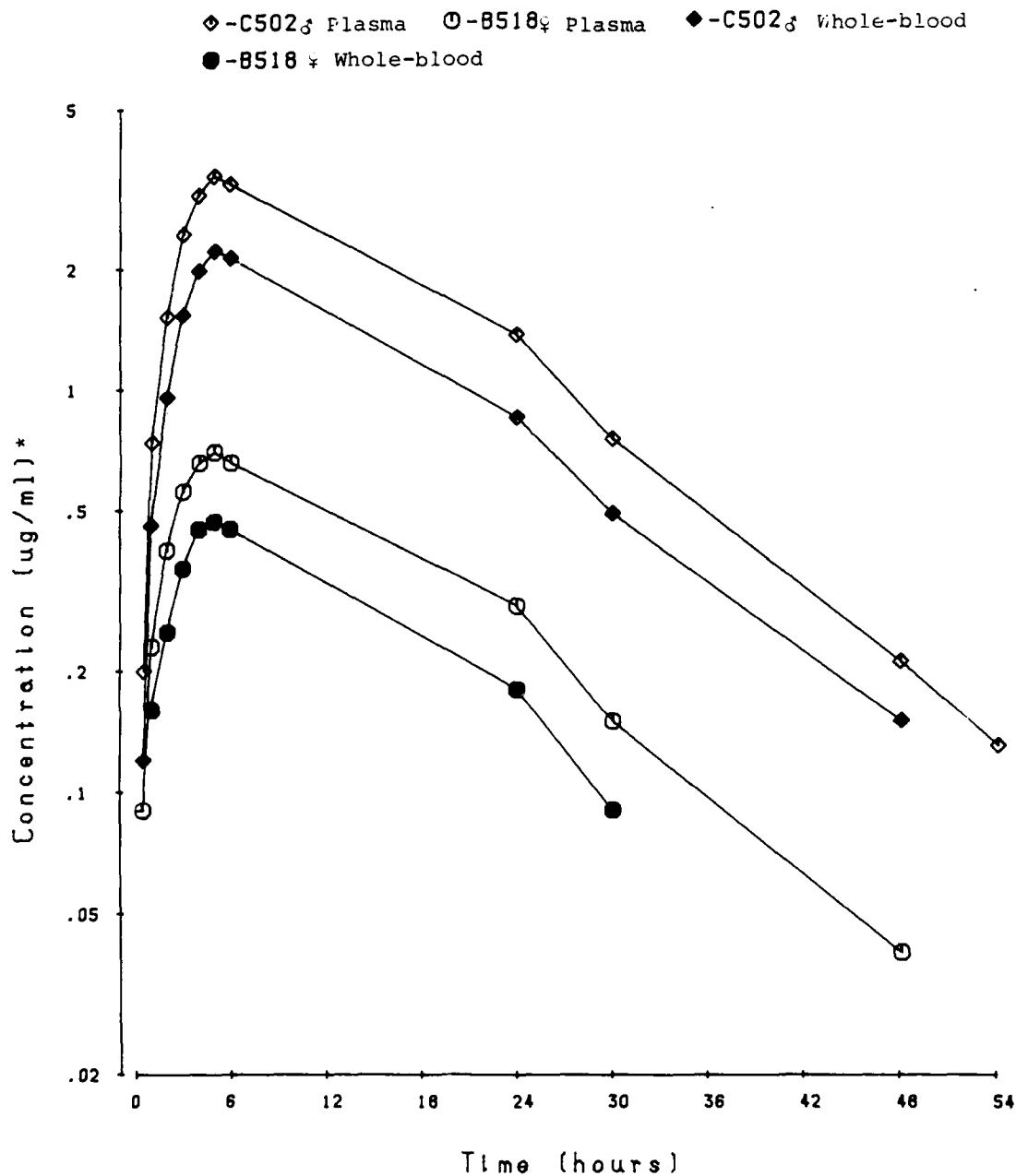


FIGURE 5

Concentrations of radioactivity in the plasma and whole-blood of monkeys with cannulated bile ducts after oral doses of  $^{14}\text{C}$ -WR 158,122



\* Concentrations of whole-blood are measured in ug/g

FIGURE 6

Mean concentrations of radioactivity in the plasma of monkeys after oral doses of  $^{14}\text{C}$ -WR 158,122, with and without incorporation of bile salts in the dose suspension

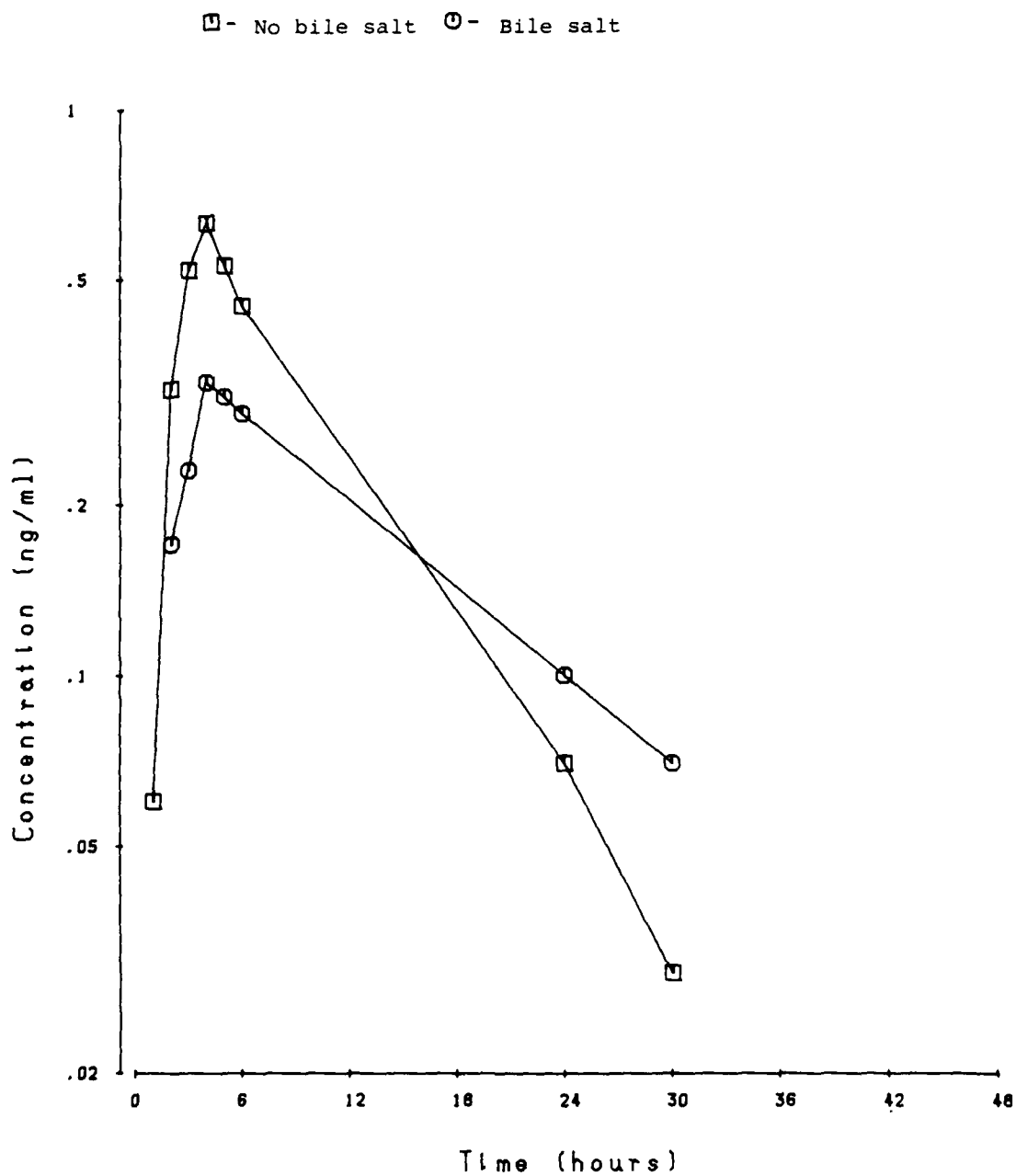


FIGURE 7

Observed and fitted curves for concentrations of total radioactivity in plasma of a male monkey (intact, C5028) after a single oral dose of  $^{14}\text{C}$ -WR 158,122

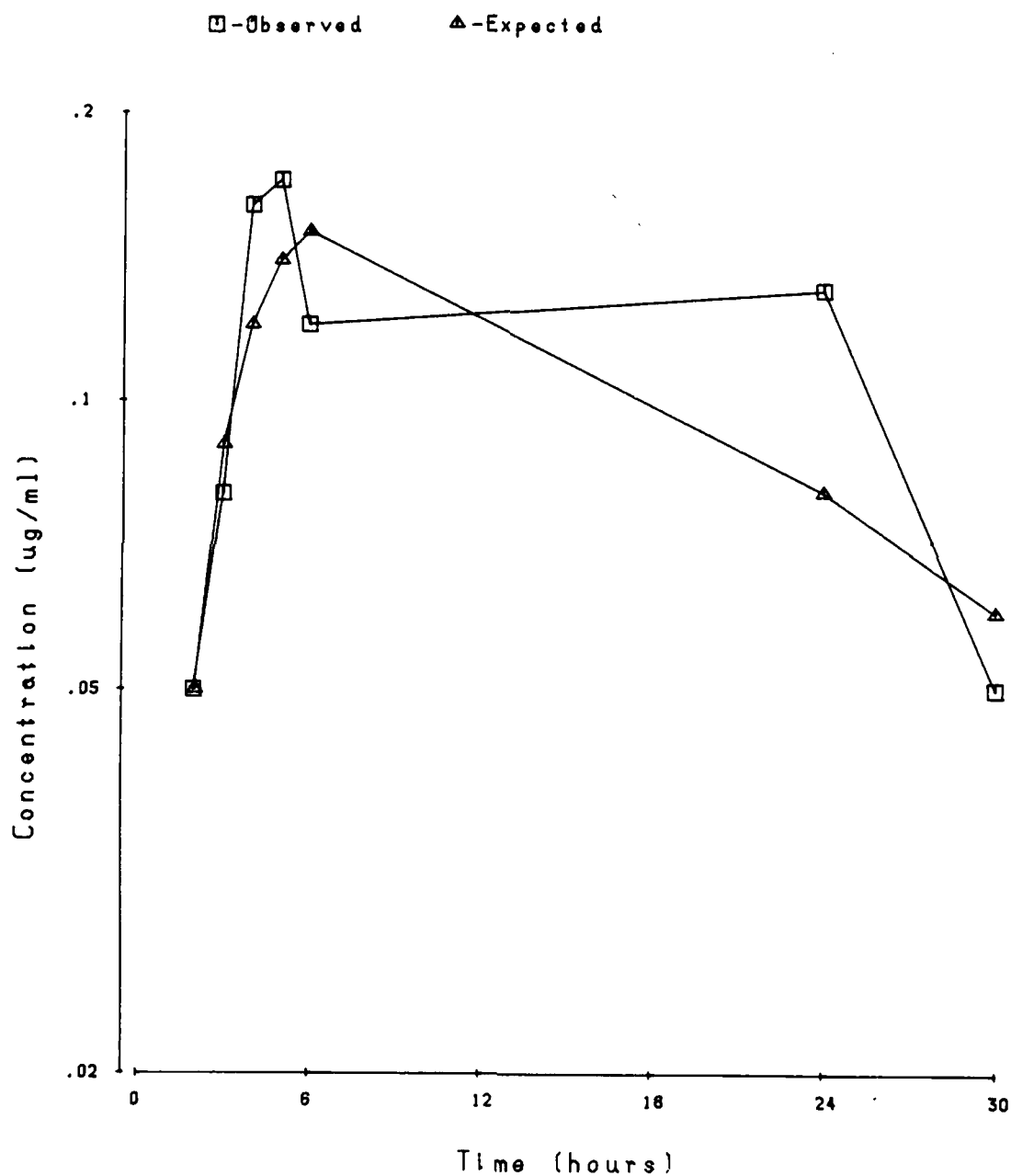




FIGURE 8

Observed and fitted curves for concentrations of total radioactivity in plasma of a female monkey (intact, B518♀) after a single oral dose of  $^{14}\text{C}$ -WR 158,122

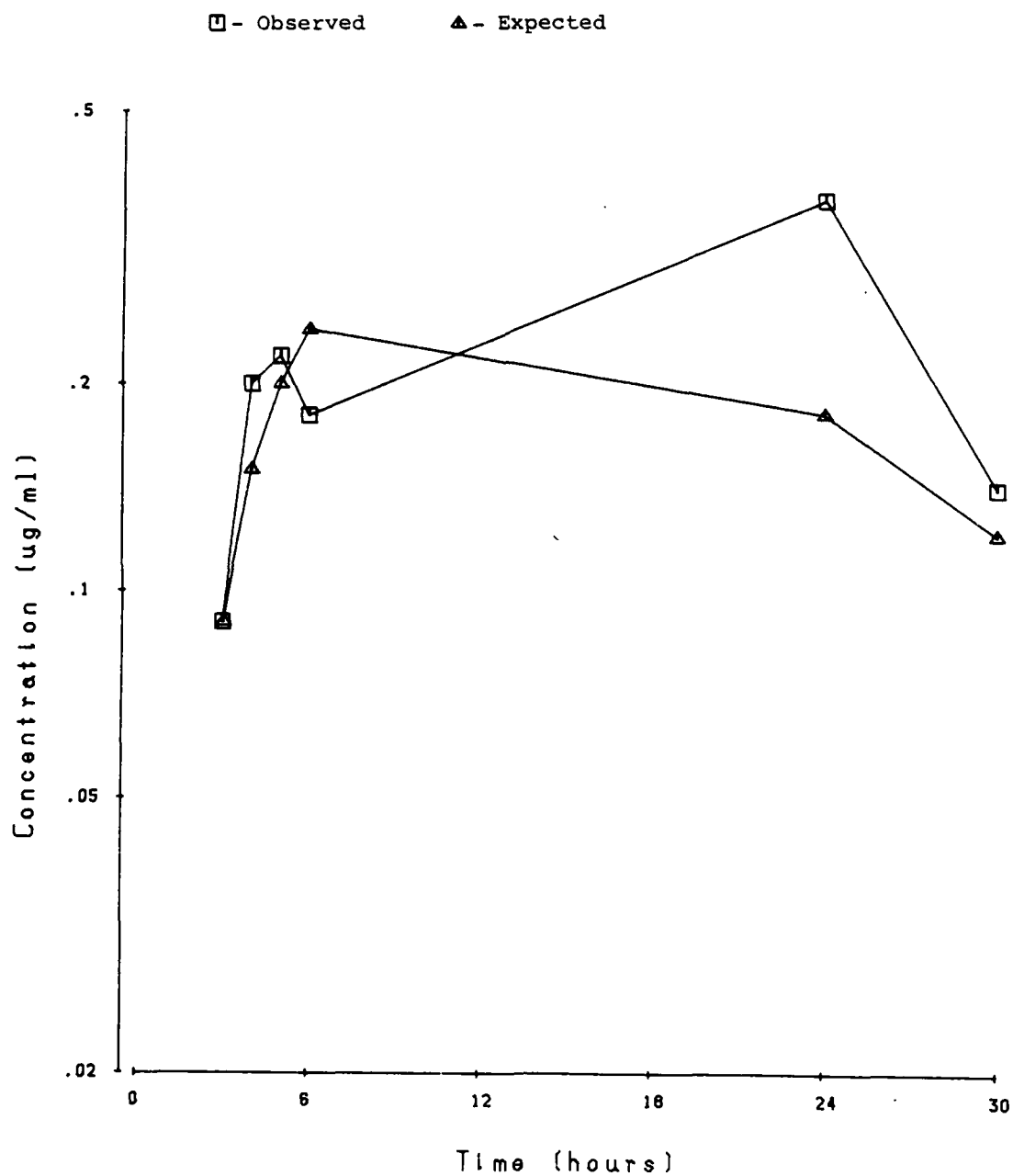


FIGURE 9

Observed and fitted curves for concentrations of total radioactivity in plasma of a male monkey (bile duct cannulated, C5023) after a single oral dose of  $^{14}\text{C}$ -WR 158,122

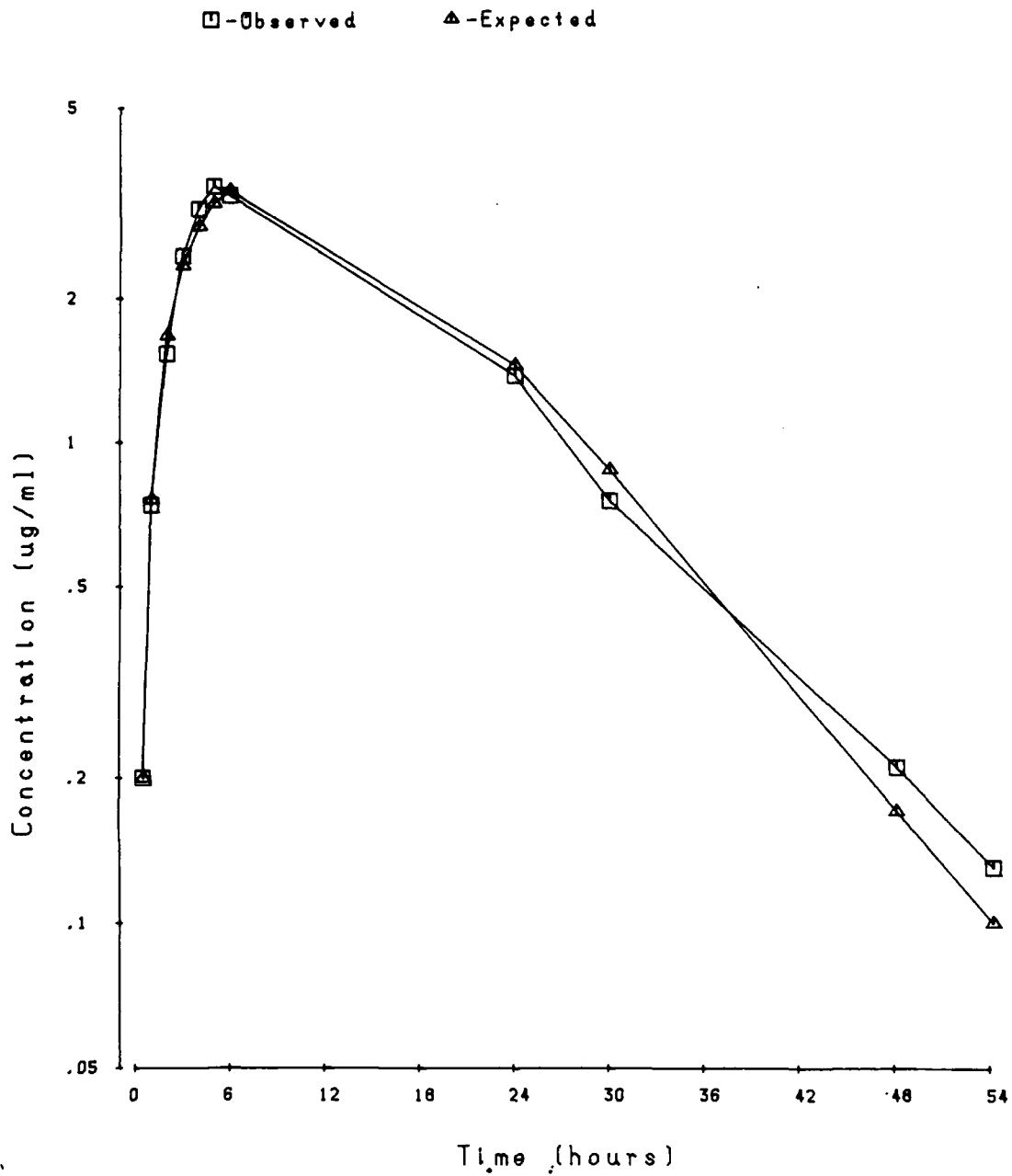


FIGURE 10

Observed and fitted curves for concentrations of total radioactivity in plasma of a female monkey (bile duct cannulated, B518\*) after a single oral dose of  $^{14}\text{C}$ -WR 158,122

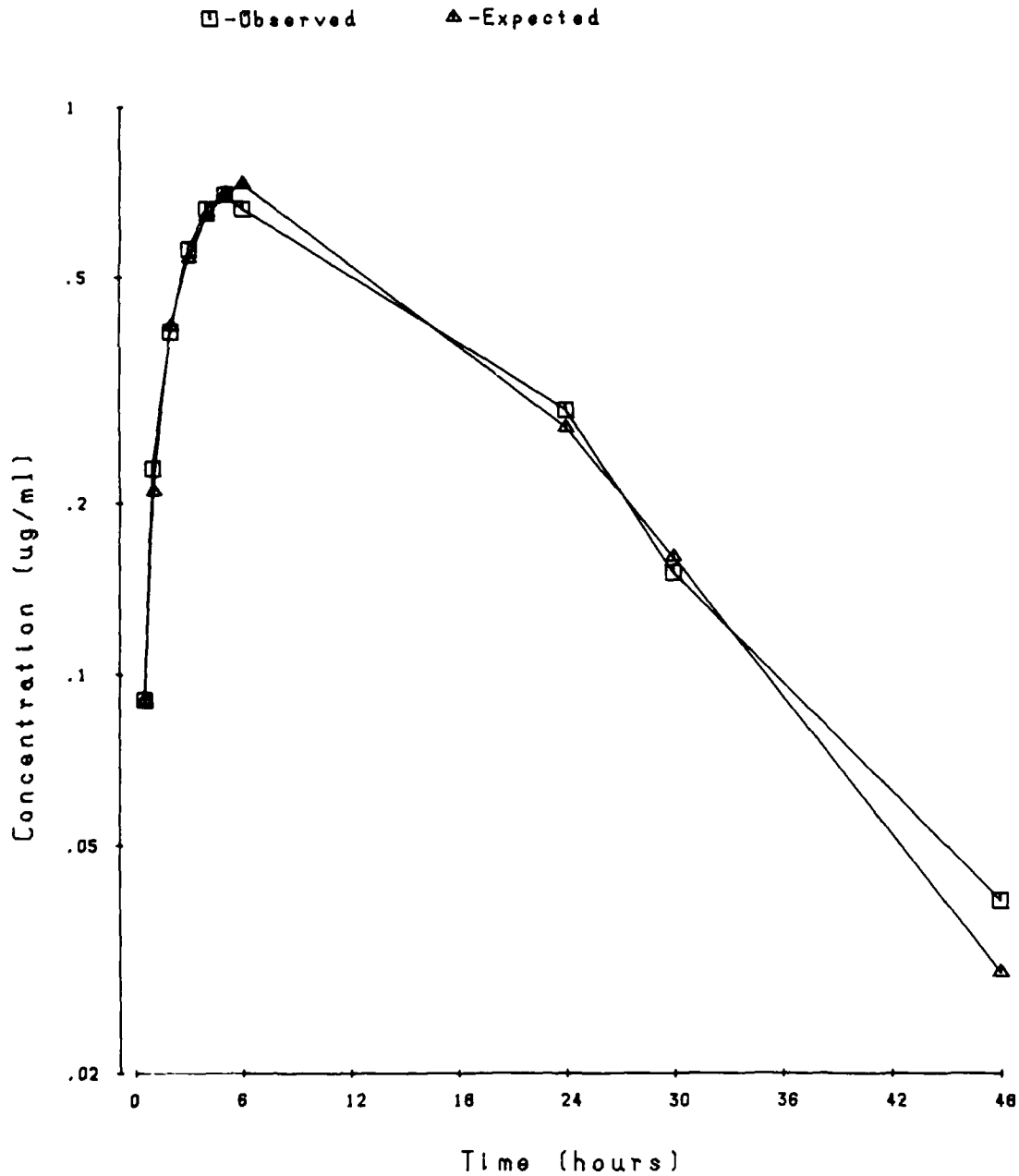


FIGURE 11

Observed and fitted concentrations of radioactivity in the plasma of a monkey (C4984) after a single oral dose of  $^{14}\text{C}$ -WR 158,122 with no incorporation of bile salts in the dose suspension

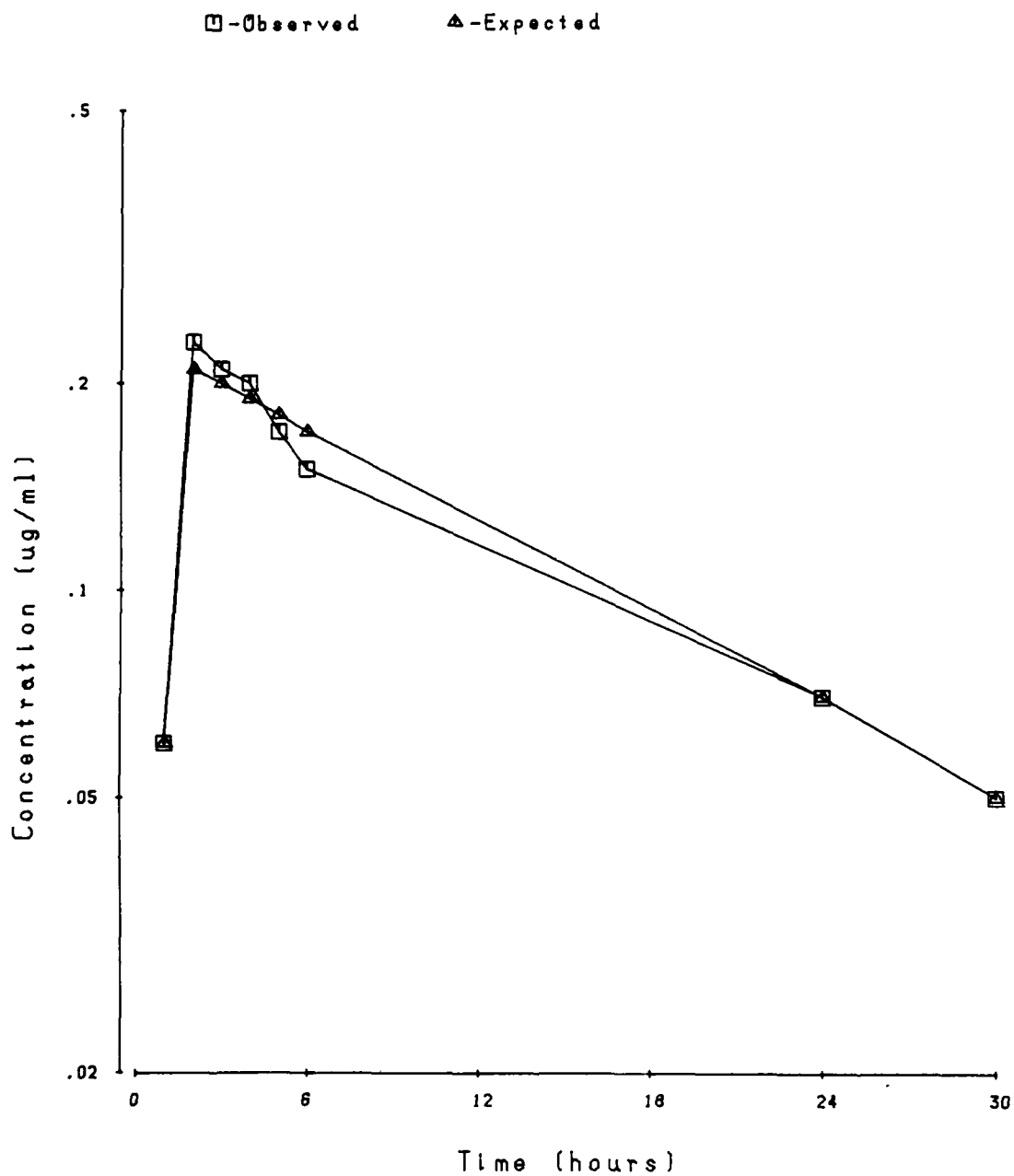


FIGURE 12

Observed and fitted concentrations of radioactivity in the plasma of a monkey (C505♀) after a single oral dose of  $^{14}\text{C}$ -WR 158,122 with no incorporation of bile salts in the dose suspension

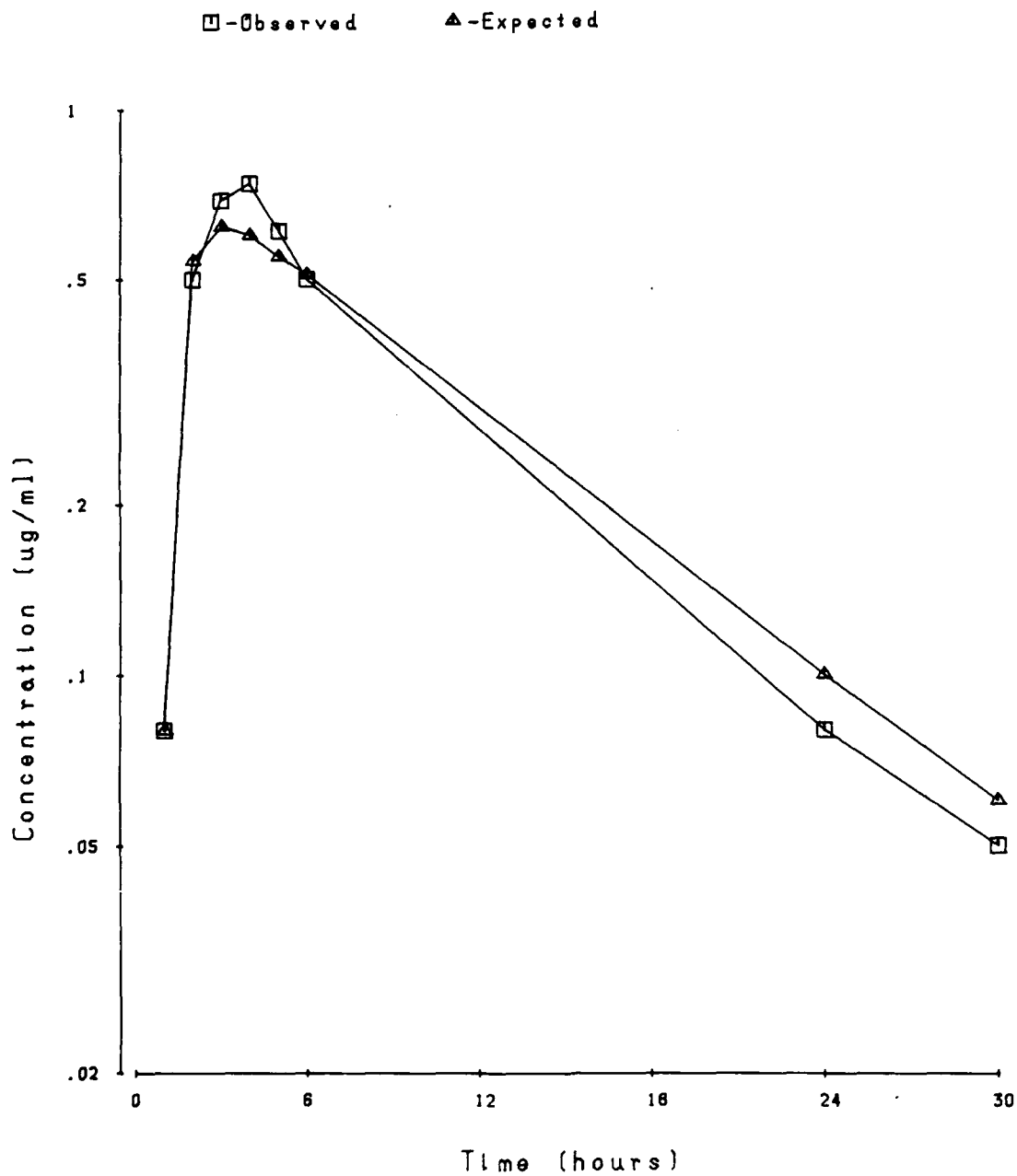


FIGURE 13

Observed and fitted concentrations of radioactivity in the plasma of a monkey (343\*) after a single oral dose of  $^{14}\text{C}$ -WR 158,122 with no incorporation of bile salts in the dose suspension

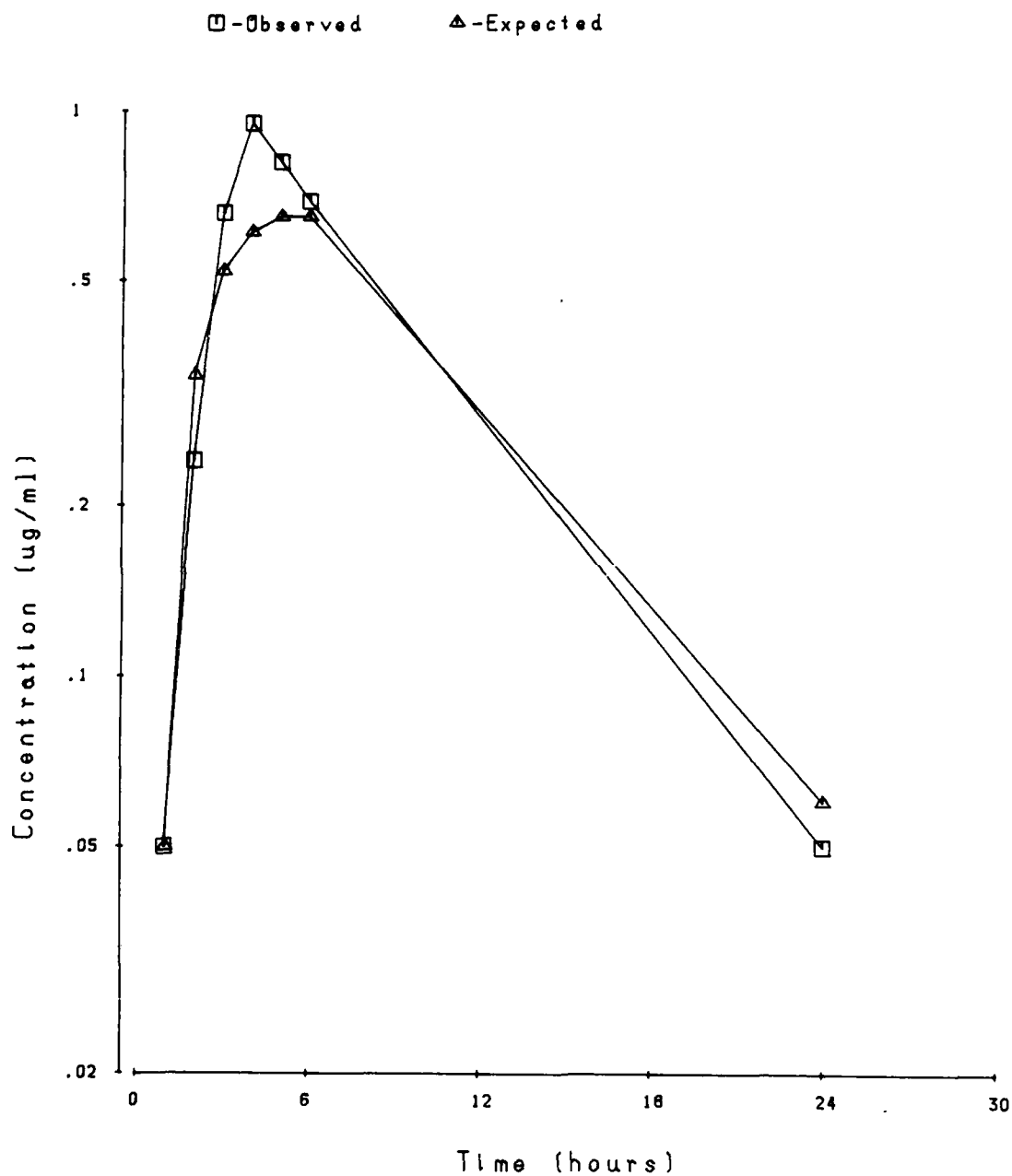


FIGURE 14

Observed and fitted concentrations of radioactivity in the plasma of a monkey (C4989) after a single oral dose of  $^{14}\text{C}$ -WR 158,122 with incorporation of bile salts in the dose suspension

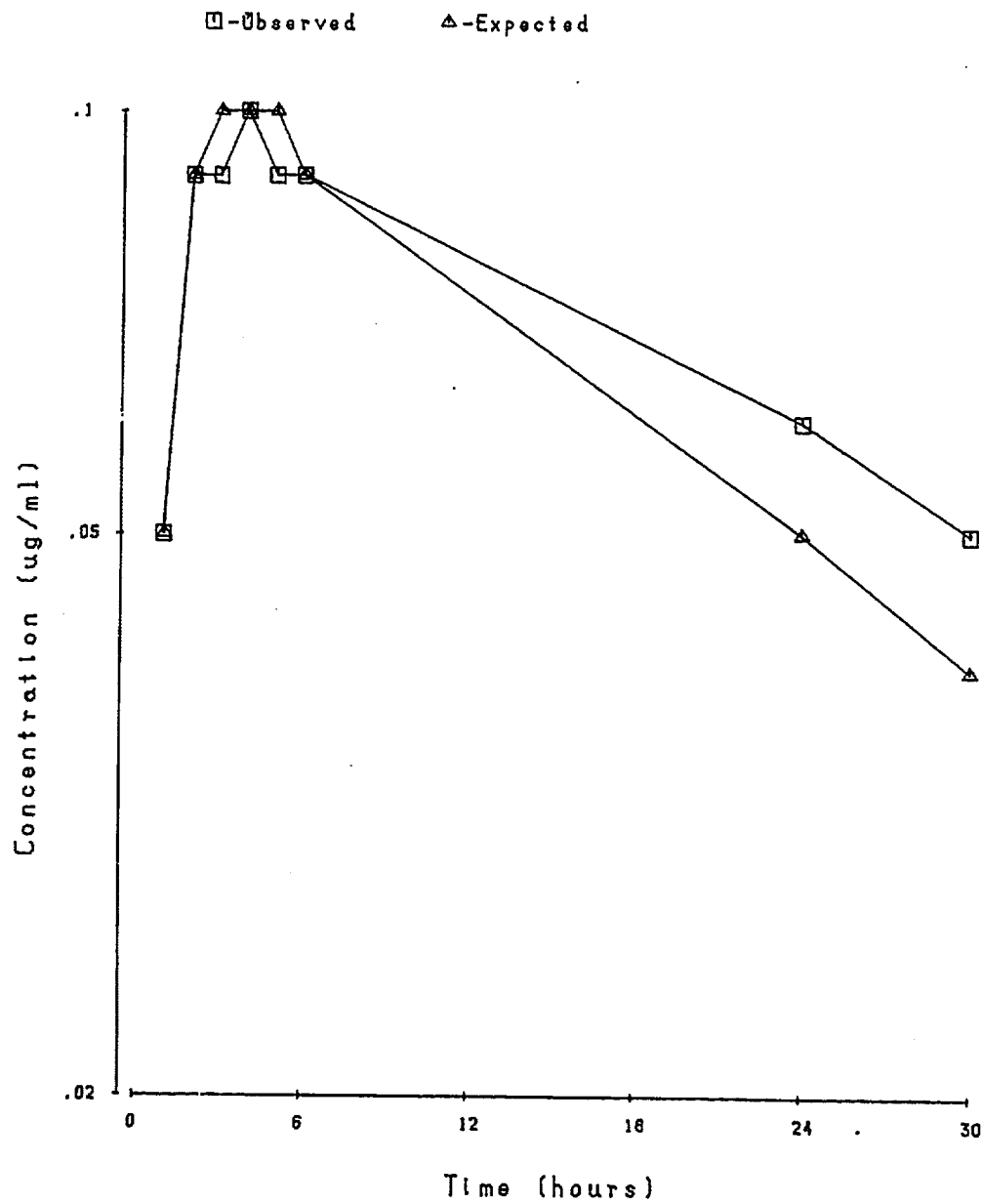


FIGURE 15

Observed and fitted concentrations of radioactivity in the plasma of a monkey (C505\*) after a single oral dose of  $^{14}\text{C}$ -WR 158,122 with incorporation of bile salts in the dose suspension

KEY:  $\square$ -Observed  $\triangle$ -Expected

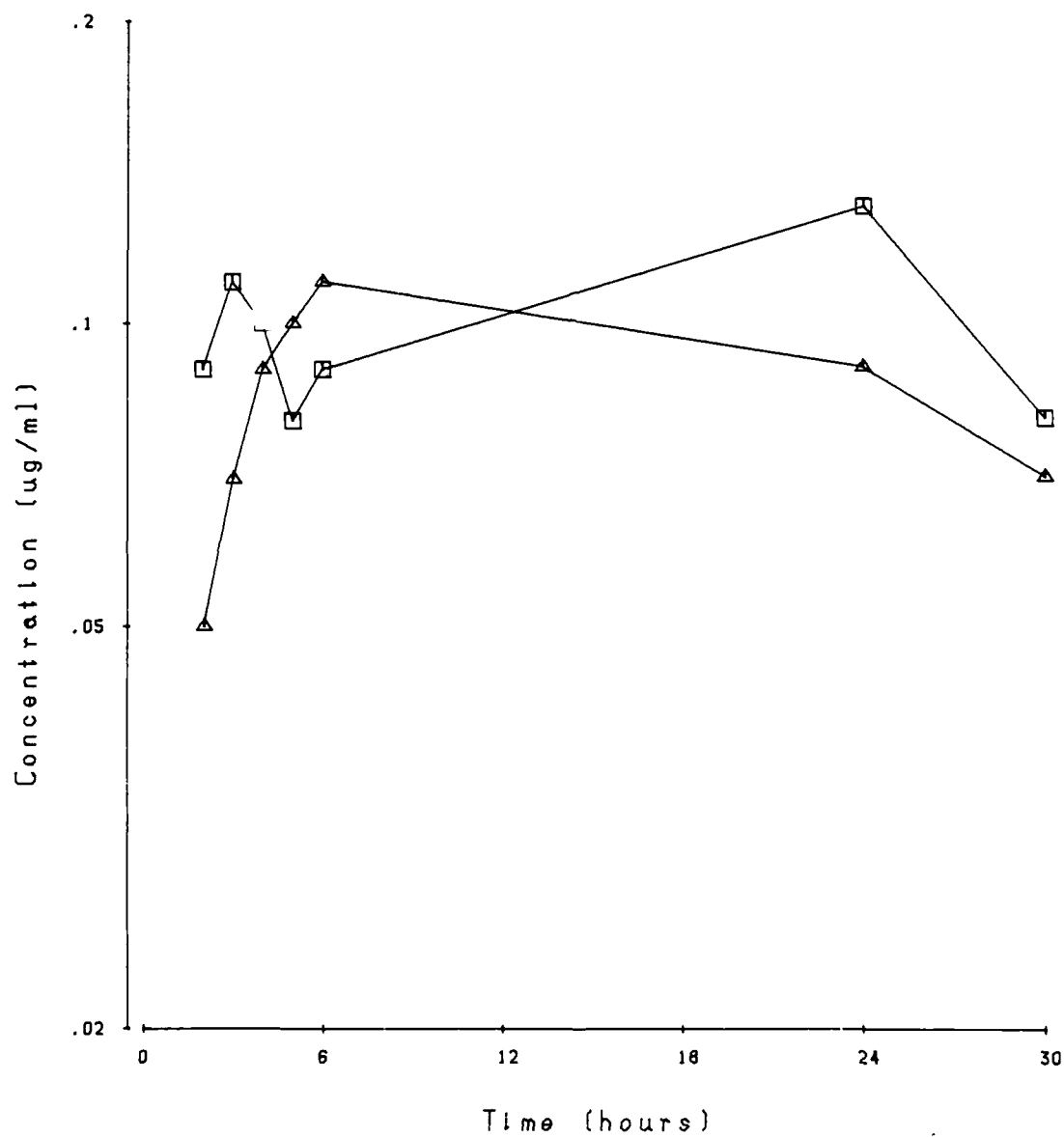
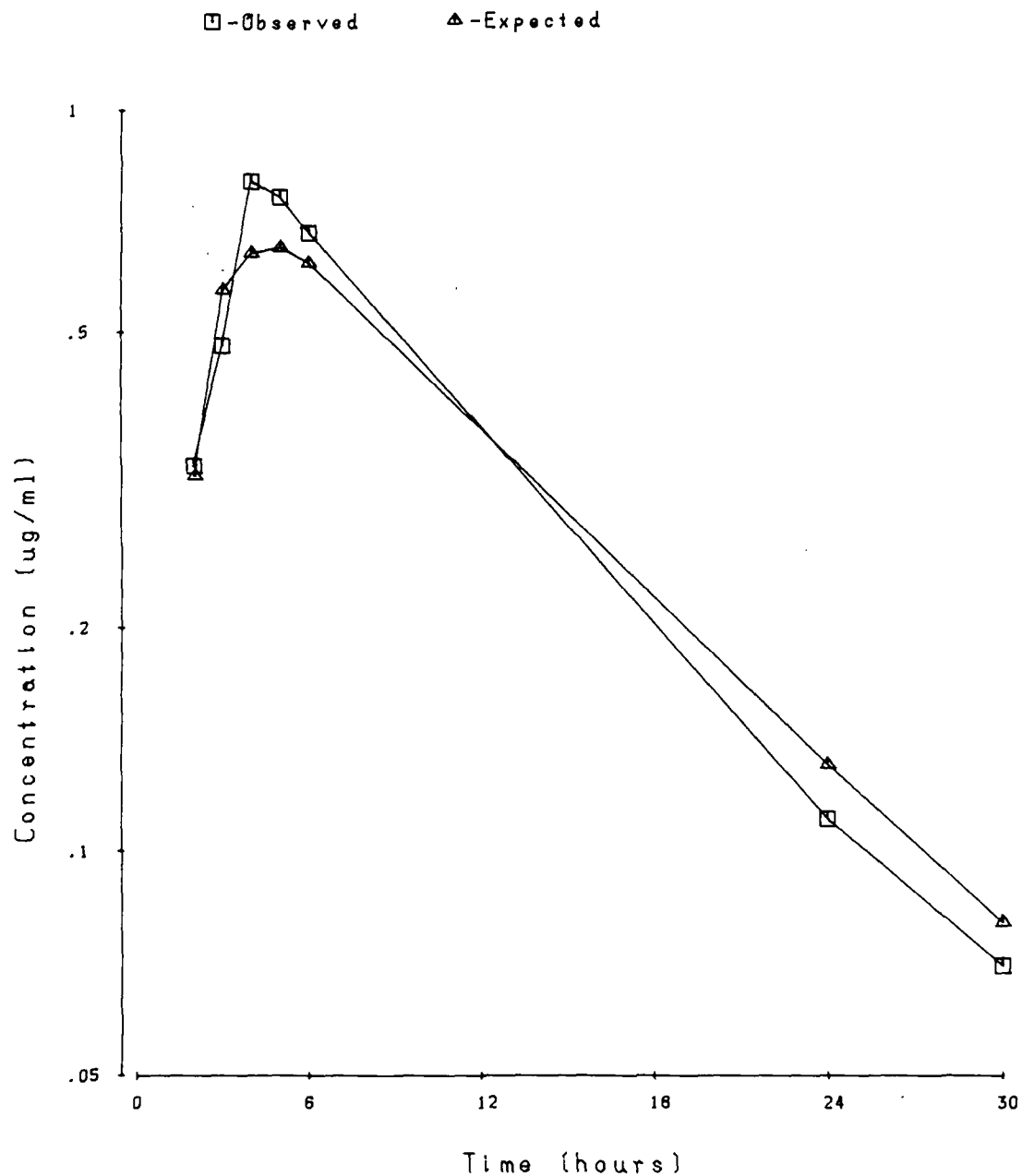




FIGURE 16

Observed and fitted concentrations of radioactivity in the plasma of a monkey (343\*) after a single oral dose of  $^{14}\text{C}$ -WR 158,122 with incorporation of bile salts in the dose suspension



THE IDENTIFICATION OF THE MAJOR  
METABOLITES OF  $^{14}\text{C}$ -WR 158,122 IN THE  
RHESUS MONKEY

## SUMMARY

1. The major metabolites of WR 158,122 in urine and bile of rhesus monkeys receiving 5 mg/kg oral doses have been isolated and purified using a combination thin-layer and high performance liquid chromatography.

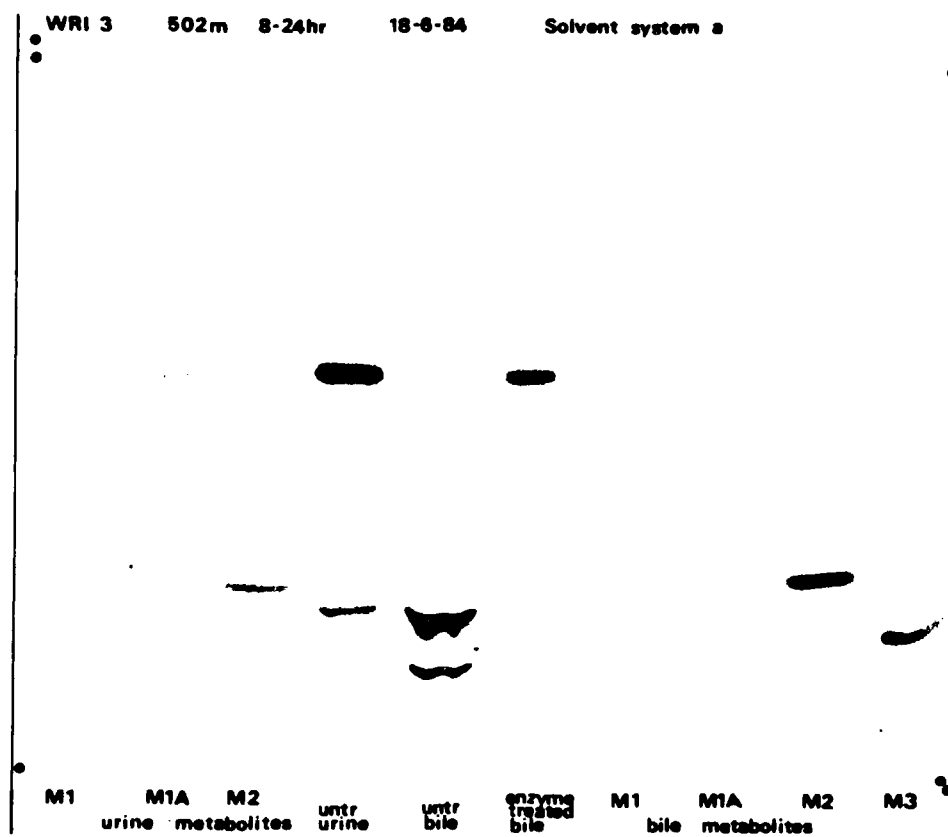
Bile metabolites were present mainly in the form of conjugates and samples were incubated with  $\beta$ -glucuronidase/sulphatase prior to isolation.

2. The isolated metabolites were investigated by mass spectrometry as their trimethylsilyl derivatives. The major metabolite in urine and bile was assigned as a dihydrodiol presumably resulting from epoxidation of the naphthalene ring. A second minor component of similar chromatographic polarity appeared to be an isomeric dihydrodiol giving an almost identical mass spectrum. This isomer could be formed via epoxidation at a different position in the naphthalene ring.

A more polar metabolite also isolated from bile and urine has been assigned to a diphenolic compound which could be formed by dehydrogenation of the dihydrodiol. A further related diphenolic metabolite, only isolated from bile, was tentatively assigned to a compound derived by oxidative deamination to give a quinazolinone.

FIGURE 1

Thin-layer autoradiograph of isolated urinary and biliary metabolites obtained after oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey. The developing solvent system was isopropanol : 35% w/v aqueous ammonia (8 : 2,v/v)



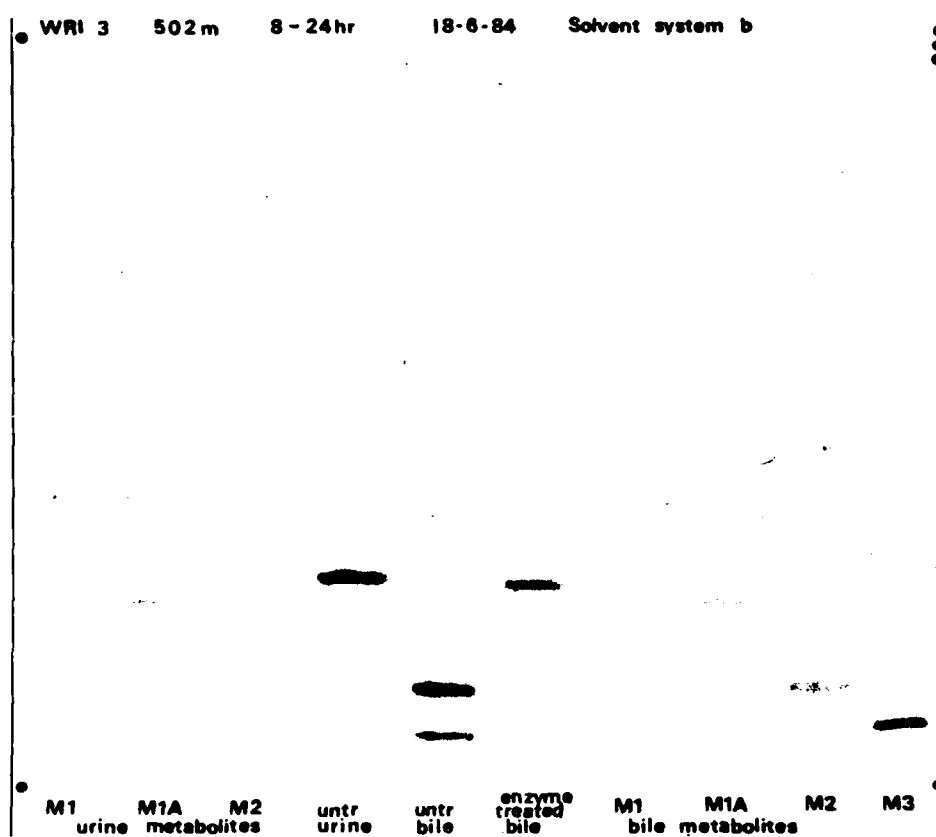
Approx.  
R<sub>f</sub> values

0.51    0.23

0.5    0.51    0.24    0.17

FIGURE 2

Thin-layer autoradiographs of isolated urinary and biliary metabolites obtained after oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey. The developing solvent system was chloroform : methanol : water : formic acid (80 : 25 : 3 : 3, v/v)



Approx.  
R<sub>f</sub> values

0.25    0.22    0.11

0.25    0.22    0.11    0.07

FIGURE 3

High performance liquid chromatograms (u.v. detection 250 nm) of metabolite 'M1' after oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey. (a from urine; b from bile; c from bile and urine combined). The eluting solvent system was methanol : water (1 : 1,v/v) and the retention time for 'M1' was 4.5 mins

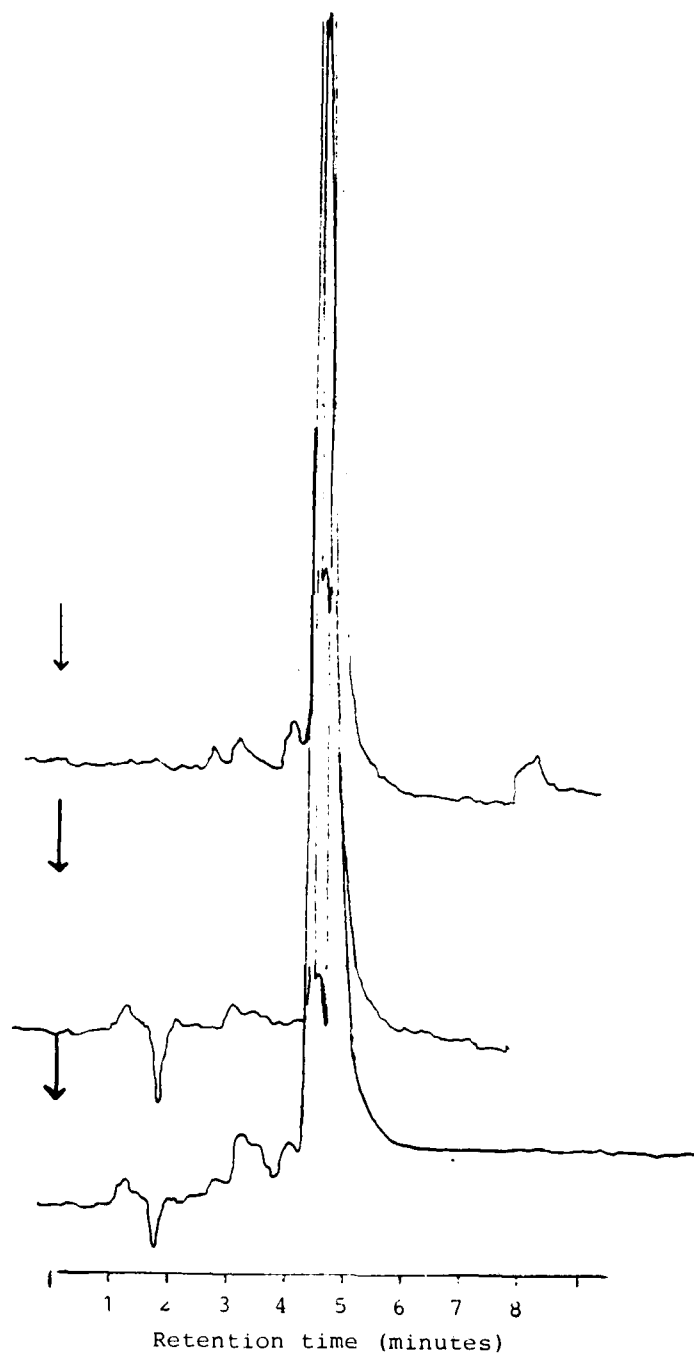


FIGURE 4

High performance liquid chromatograms (u.v. detection 250 nm) of metabolite 'M1A' after oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey. (a from urine; b from bile; c from bile and urine combined). The eluting solvent system was methanol : water (1 : 1,v/v) and the retention time for 'M1A' was 3.8 mins

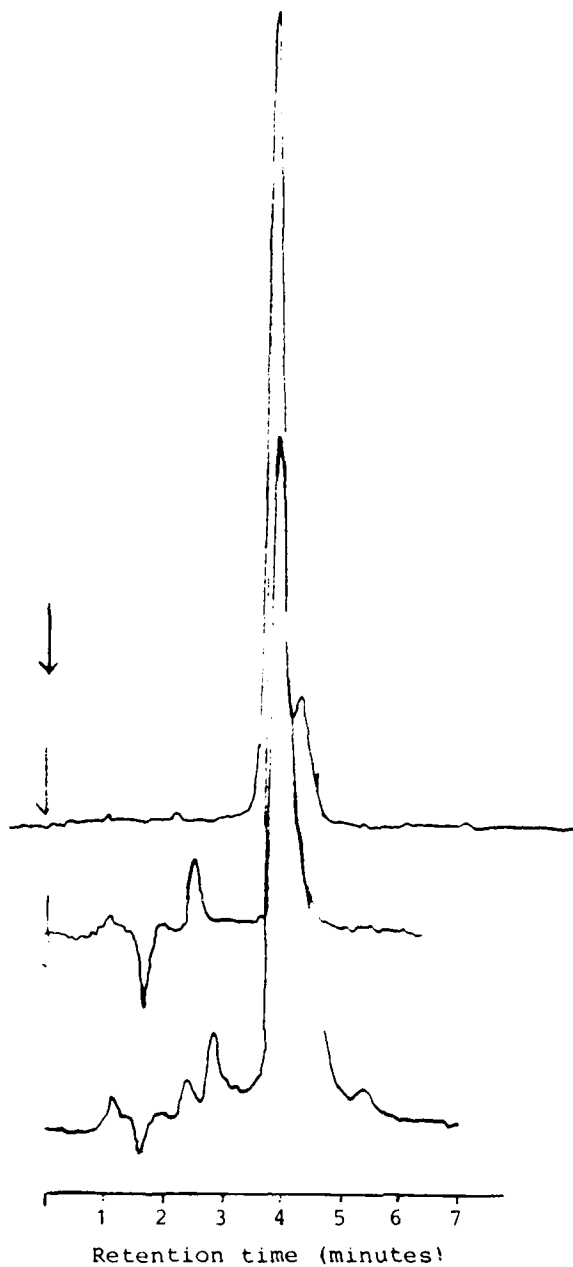


FIGURE 5

High performance liquid chromatograms (u.v. detection 250 nm) of metabolite 'M2' after oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey. (a from urine; b from bile; c from bile and urine combined). The eluting solvent system was methanol : water (1 : 3,v/v) and the retention time for 'M2' was 7.2 mins

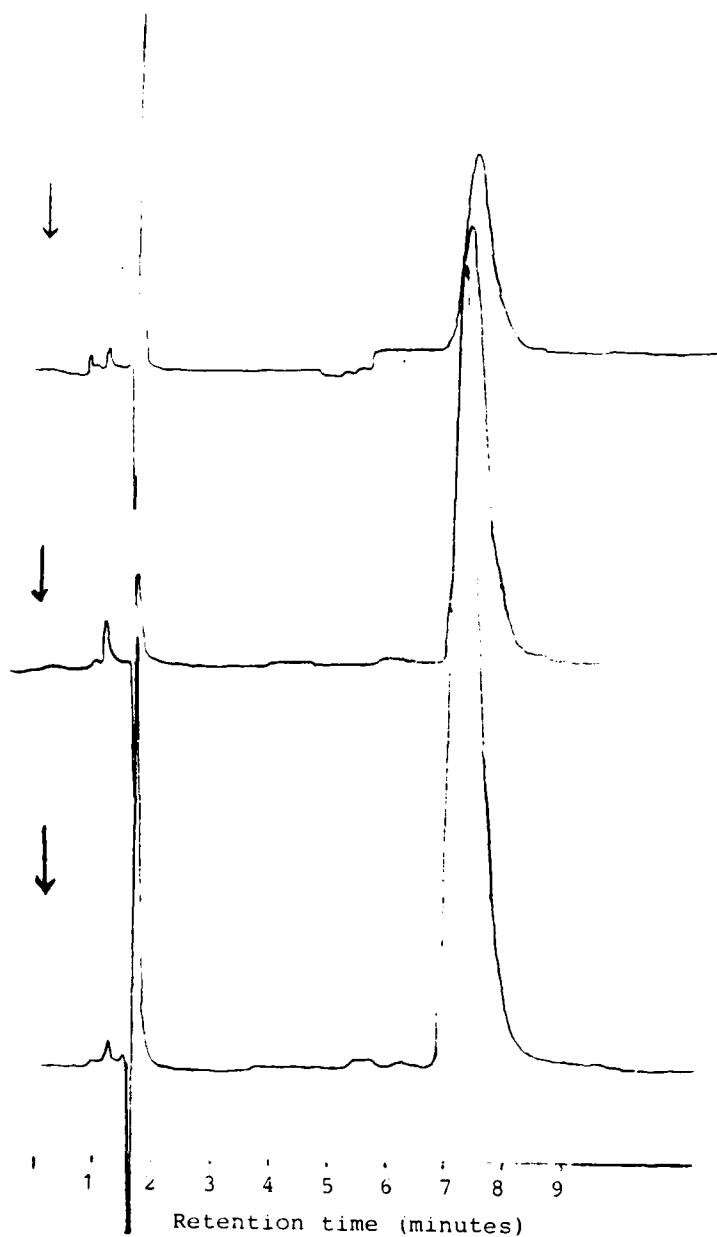




FIGURE 6

High performance liquid chromatograms (u.v. detection 250 nm) of biliary metabolite 'M3' after oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey. The eluting solvent system was methanol : water (1 : 4, v/v) and the retention time for 'M3' was 6.5 mins

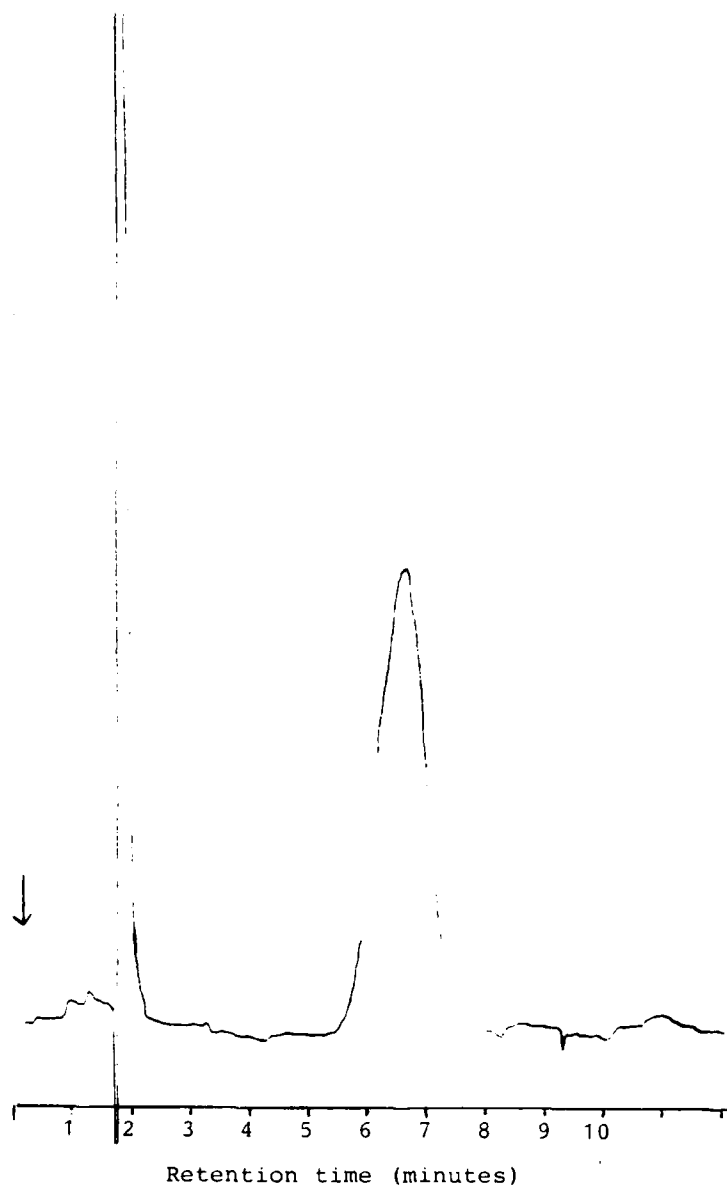


FIGURE 7

Electron impact mass spectrum of the trimethylsilyl derivative of metabolite 'M1' isolated from urine following oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey

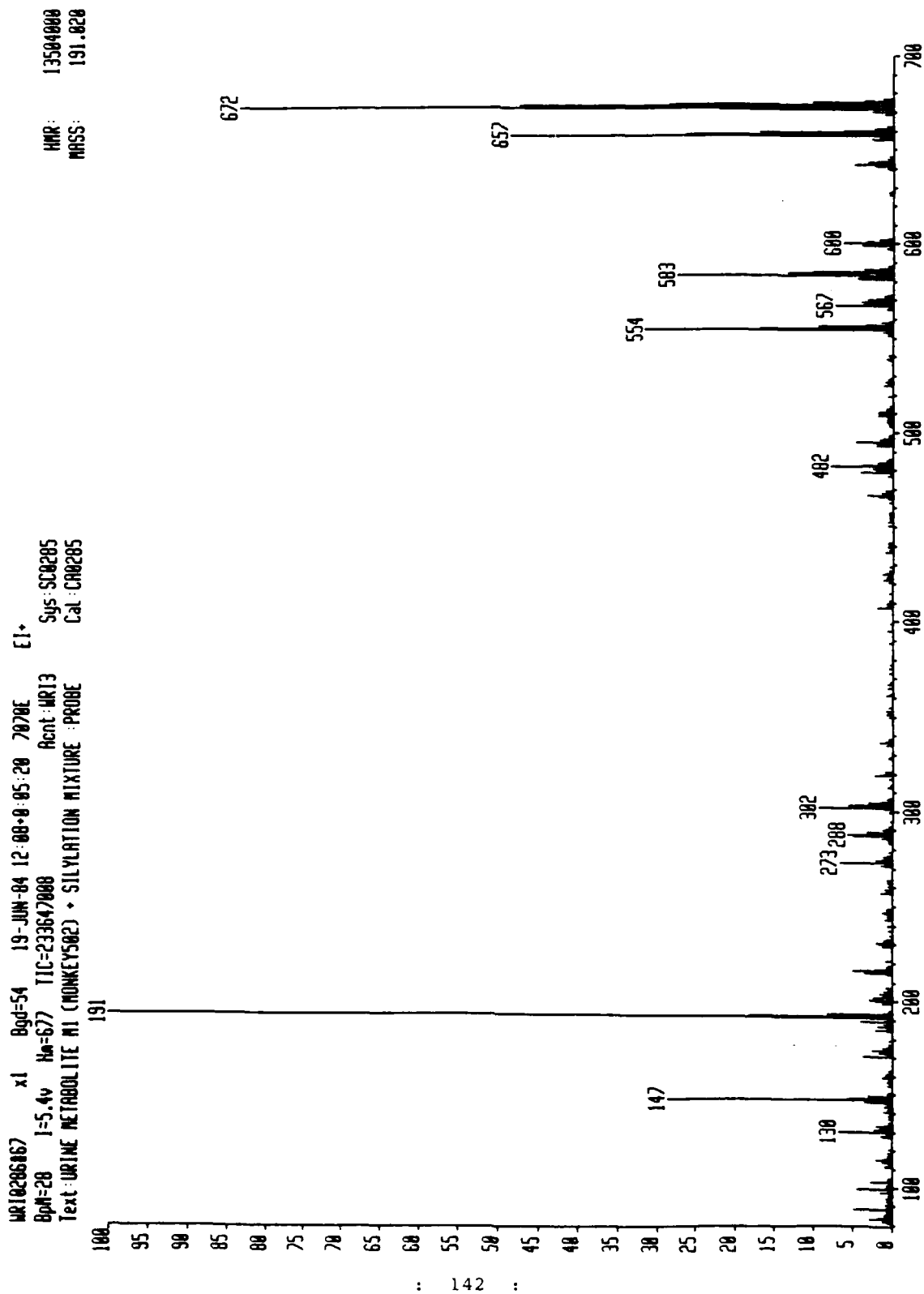


FIGURE 8

Electron impact mass spectrum of the deuterio-trimethylsilyl derivative of metabolite 'M1' isolated from urine following oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey

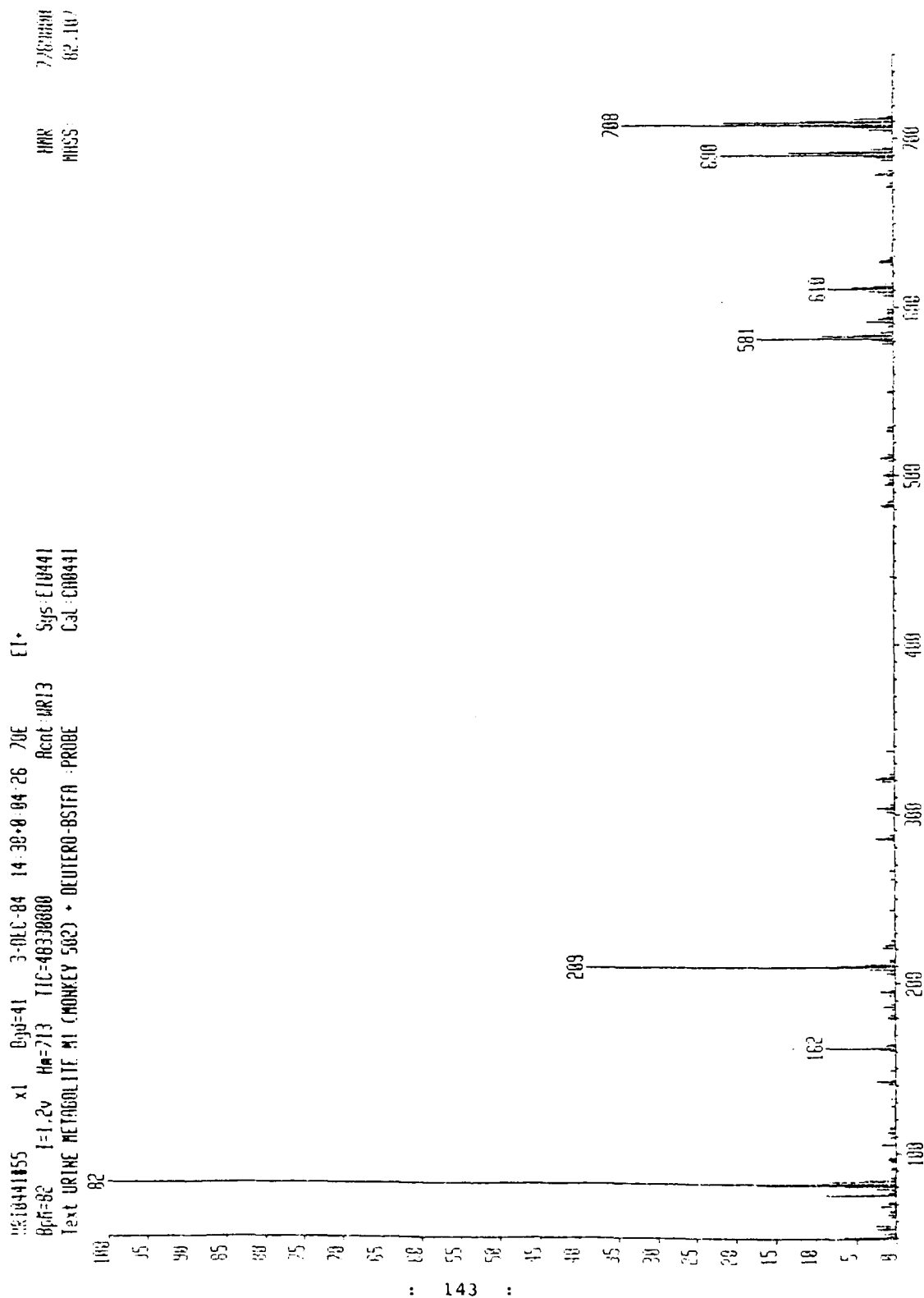


FIGURE 9

Electron impact mass spectrum of the trimethylsilyl derivative of metabolite 'M1A' isolated from urine following oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey

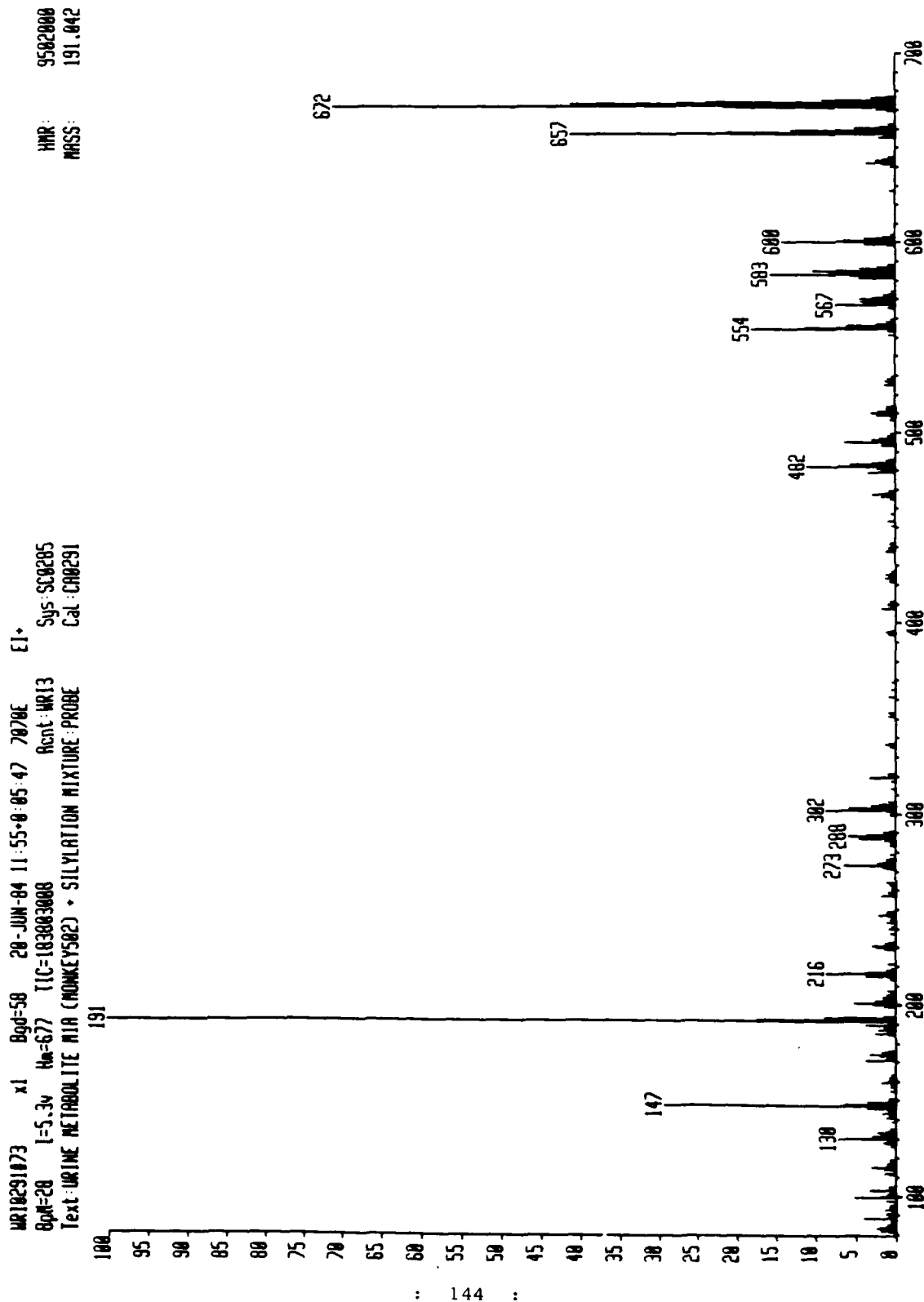


FIGURE 10

Electron impact mass spectrum of the deuterio-trimethylsilyl derivative of metabolite 'M1A' isolated from urine following oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey

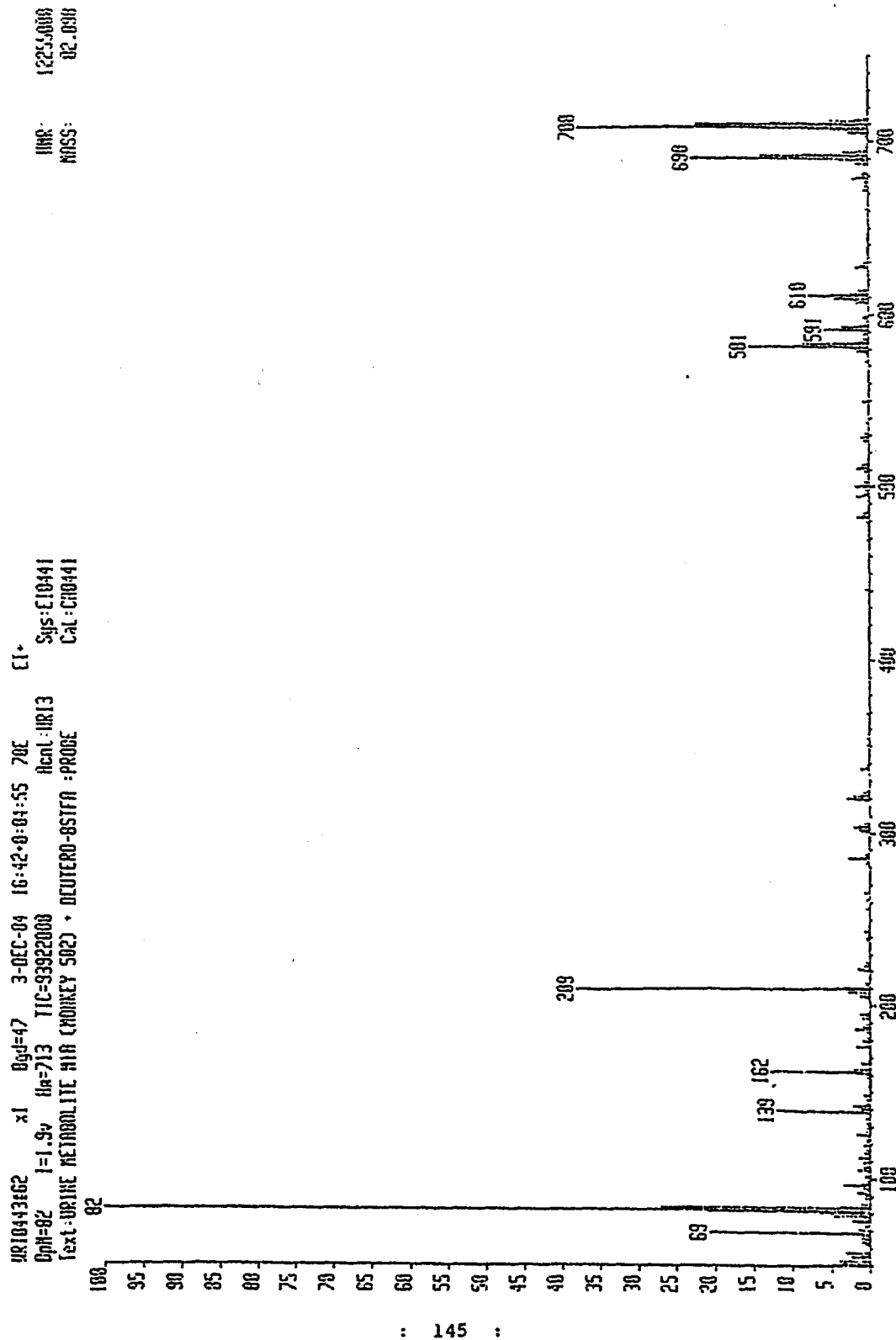


FIGURE 11

Electron impact mass spectrum of the trimethylsilyl derivative of metabolite 'M2' isolated from urine following oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey

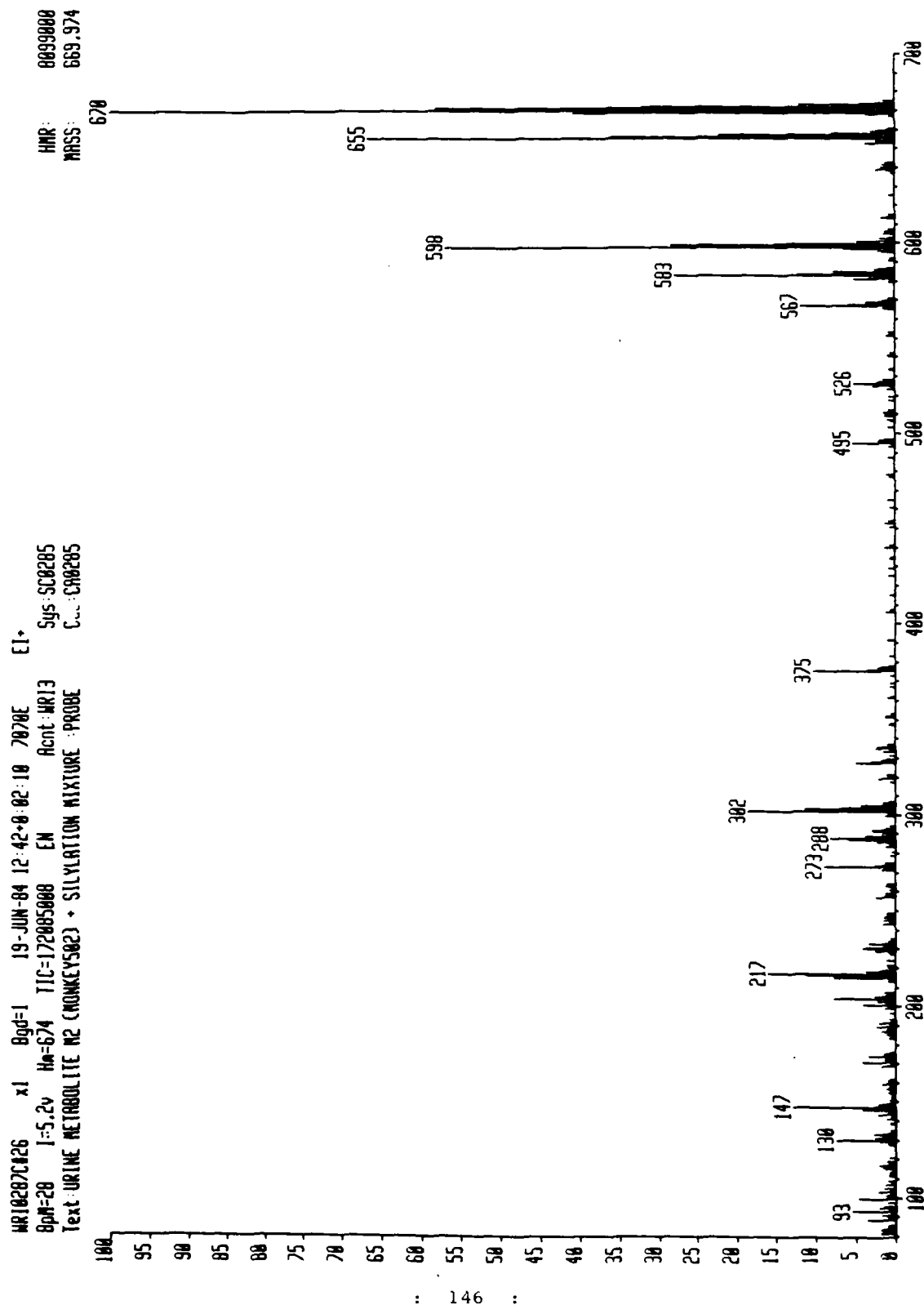


FIGURE 12

Electron impact mass spectrum of the trimethylsilyl derivative of metabolite 'M3' isolated from bile following oral administration of  $^{14}\text{C}$ -WR 158,122 to a male rhesus monkey

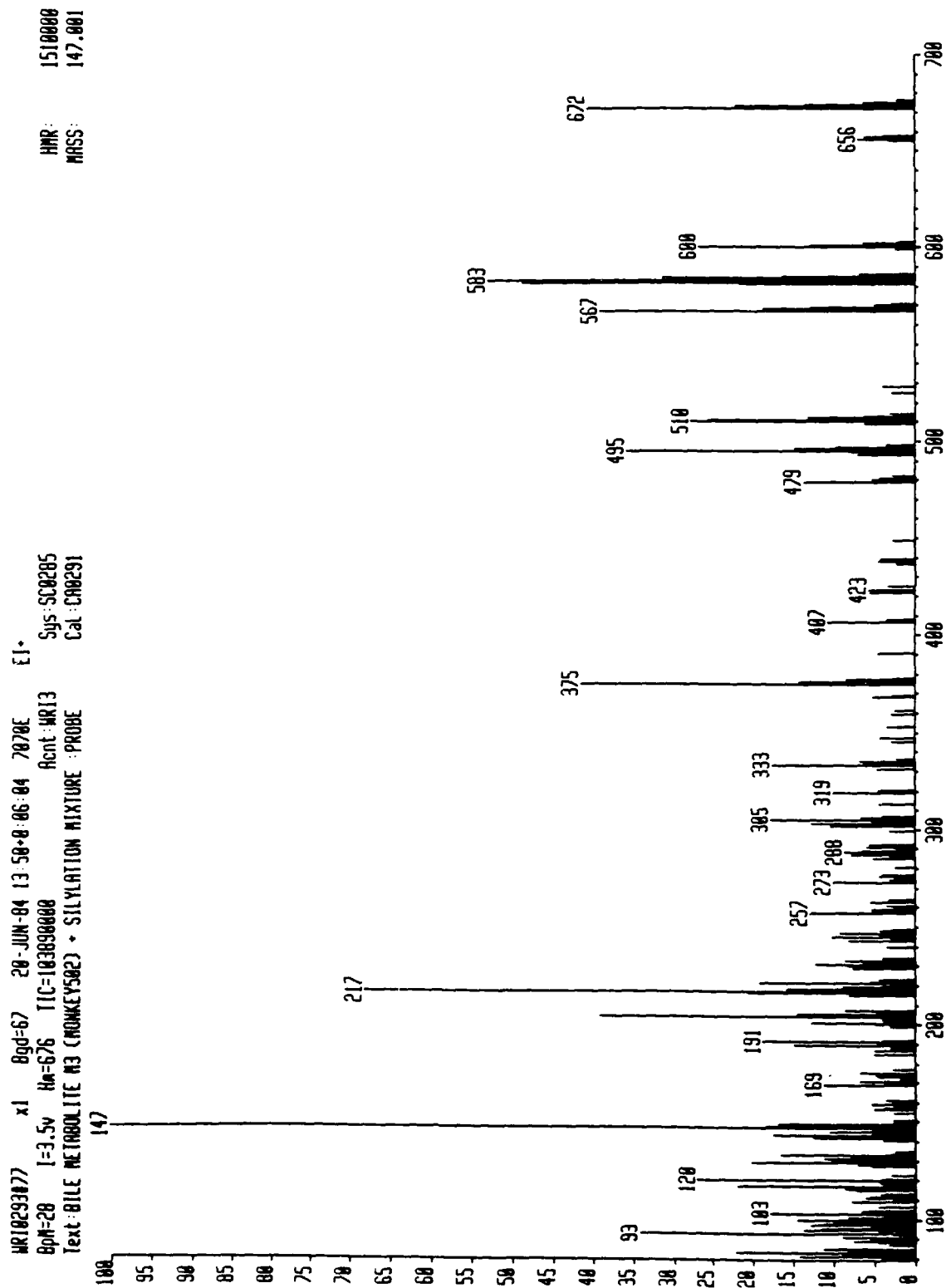
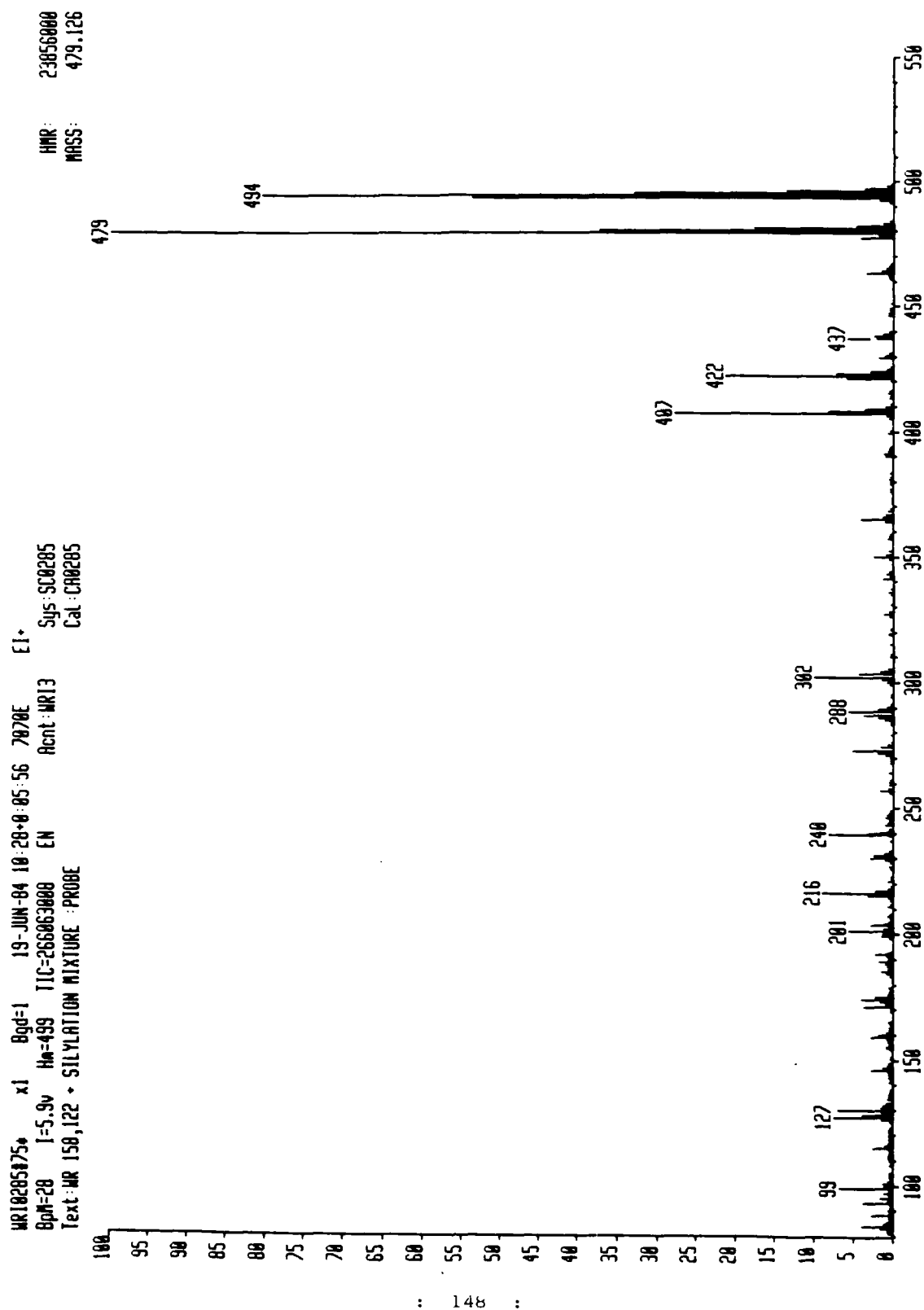


FIGURE 13

Electron impact mass spectrum of the trimethylsilyl derivative of  
 $^{14}\text{C}$ -WR 158,122





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